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LABORATORY MANUAL
FOR THE
DETECTION OF POISONS
AND
POWERFUL DRUGS

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AUTHORIZED TRANSLATION

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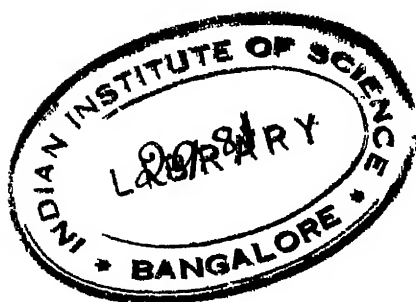
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AUTHOR'S PREFACE

In the latest edition of this book, the original arrangement of subject-matter has been retained but each chapter has undergone thorough revision. Several new subjects and methods have been incorporated and the number of illustrations has been considerably increased. The chemical literature has received full consideration and also, as far as seemed necessary, that relating to matters of pharmacologico-chemical interest. In rendering expert opinions, the forensic chemist in many instances will find these references to original literature very useful and by studying them students should acquire a far better knowledge of the subject. Information with regard to the physiological action of the more important poisons and drugs should prove of service to many chemists and the same may be said of the matter pertaining to the changes which these substances undergo in the animal body and to the forms of combination in which they are eliminated. Students should not be permitted to perform their laboratory work in a purely perfunctory manner but they should be encouraged to take advantage of every opportunity to acquire at the same time a theoretical knowledge of the subject by studying methods of preparation and the chemistry of the more important substances, the alkaloids in particular.

The new edition of this work, in comparison with what has gone before, is much more extensive in its scope but the subject-matter is so distributed as not to be detrimental in any way to the book as a whole. The student in most instances will not be concerned with more than the first three chapters, although a student of pharmacy may also add the sixth chapter dealing with the methods used in assaying potent drugs and their preparations.

WILHELM AUTENRIETH.

FREIBURG IN BREISGAU.

TRANSLATOR'S PREFACE TO THE SIXTH EDITION

This edition of "*Detection of Poisons*," though differing hardly at all from earlier editions in method of treatment, presents notable changes as far as contents are concerned. Many new substances and methods have found places in its pages. The first chapter which is confined to volatile poisons now includes several related phenols in addition to carbolic acid, and other compounds appearing here for the first time are butyl chloral hydrate, isoamyl alcohol, formaldehyde and formic acid. Added to the non-volatile organic substances, which go to make up the second chapter, are lactophenine, salol, aspirine, and the alkaloid scopolamine. The third chapter treating of metallic poisons has undergone practically no change.

By far the greater part of the new matter is to be found in the last three chapters of the book. In the fourth chapter, which is concerned with such substances as find no place in the first three groups of poisons, the author has introduced such inorganic compounds as hydrofluoric acid and fluorides, bromine and bromides, iodine and iodides, hydrogen sulphide, sulphur dioxide and sulphurous acid, boric acid, nitrous gases and nitrites. Salvarsan and derivatives are appropriate additions to the organic arsenicals. Synthetic hypnotics and sedatives, represented by such compounds as propanal, luminal, medinal, dial, adalene, bromural, neuronal, diogenal and nirvanol, have received attention. Aconitine, pseudo-aconitine, arecoline, johimbine and lobeline are selections from the less common alkaloids. Such cocaine substitutes as novocaine, tutocaine, psicaine, orthoform (new), and stovaine have been included together with the morphine derivatives, dionine and heroine. The subject of digitalis glucosides has received fuller treatment and been supplemented by the addition of the related strophanthin. Even the unusual toxic substances isolated by Wieland from the skin of the toad have been made a part of this lengthy chapter.

Certain apparatus and special analytical methods form the subject-matter of the fifth chapter. Different forms of extractors

are described and particular attention is paid to colorimetric estimations by means of the author's colorimeter. The usefulness of microsublimation *in vacuo* as a means of detecting alkaloids is emphasized. Special methods of analysis are divided into two groups. The first group comprises methods applicable to the examination of inorganic substances such as phosphorus, arsenic and certain metals. The second group deals with the examination of organic substances among which are hydrocyanic acid, methyl alcohol, formaldehyde, several important alkaloids, and chloral, carbolic acid, pyrimidine and veronal as found in urine. In conclusion this chapter takes up methods of examining inflammables containing phosphorus and official German methods employed in detecting arsenic and tin in textile materials.

As in former editions the sixth chapter is given over to quantitative methods of pharmaceutical interest. Many methods of the German Pharmacopoeia employed in estimating alkaloids are given in full. At the same time many non-official methods of estimating alkaloids and other substances, several of which appear for the first time, are included.

The author concludes with an Appendix in which he gives at considerable length the Meyer-Overton theory of narcosis, the investigation of the constitution of colchicin and colchicin by Windaus, and the results of Willstätter's study of the cleavage-products of scopalamine.

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INTRODUCTION

Upon the basis of chemical behavior during analysis, nearly all the commoner poisons and potent drugs may be put into one of the three following groups:

I Members of this group distil with steam from acid aqueous solution. Herein are included yellow phosphorus, hydrocyanic acid, carbolic acid, cresols, naphthols, chloroform, iodoform, chlorhydrate, aniline, nitrobenzene, methyl alcohol, ethyl alcohol (alcohol), amyl alcohol, formaldehyde and carbon disulphide.

II Members of this group are organic substances non-volatile with steam from acid aqueous solution. Hot alcohol containing tartaric acid extracts them from extraneous matter. Alkaloids, many glucosides and bitter principles, as well as various synthetic organic compounds used in medicine, such as antipyrine, acetanilide, phenacetine, pyramidone, sulphonal and veronal, are found here.

III All poisonous metals belong in this group

According to this classification, toxicologico-chemical analysis is divided into three distinct parts, each with its own special chemical procedure. A few poisons, such as mineral acids, caustic alkalies, potassium chlorate and oxalic acid, can not be placed in any of these three groups because of differences in solubility and other peculiarities. Such poisons can be detected only by applying special tests to the original material and sometimes by employing special methods.

In a toxicologico-chemical examination, material to be tested for all known poisons should be divided into at least four parts. This is necessary when, for example, poisoning is suspected but the autopsy and medical history of the deceased fail to furnish the chemist with *clues?* clues as to how to proceed in his chemical examination of the given material for poison. One portion should be examined for poisons volatile with steam from acid solution (I) and at the same time for metallic poisons (III). After removal of volatile substances by distillation, the residue can be tested for such metals as may be present. The second portion should be examined for organic substances, such as alkaloids, glucosides and synthetic

drugs (II) The third portion should be examined for those substances that find no place in the three main groups of poisons. Finally, a fourth portion of material should always be reserved for confirmatory tests, in case they are necessary. Such tests must be made when the first examination fails to establish with certainty the nature of the suspected poison, or when it has gone wrong. If only a limited amount of material is available, one portion may be used in testing for all three main groups of poisons. In that case the residue in the distillation-flask should be divided into two unequal portions. The larger should be tested for non-volatile organic substances (II) and the smaller, to which may be added the extraction-residue from II insoluble in alcohol containing tartaric acid, for metallic poisons (III). Also in this case it is advisable to reserve a small portion of the original material for subsequent tests.

In some instances it is impossible to conduct a toxicologico-chemical examination according to the fixed plan just outlined or by any other plan. Frequently the forensic expert cannot formulate a plan until he has taken all attendant circumstances into consideration. Before entering upon an investigation of this character, the chemist should first thoroughly and critically study all papers relating to the case, such as the medical history, if there is one, and the report of the autopsy. Particular attention should be paid to drugs administered during illness.

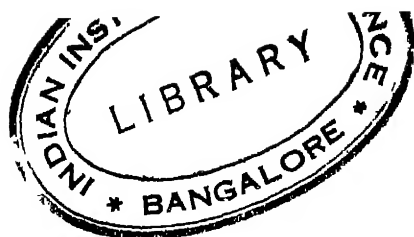
Poisons differ widely in their behavior in the animal organism. Some are retained by certain organs, such as the liver, whereas others pass into the blood and are rapidly eliminated in the urine. For these reasons it would obviously be futile in many cases to remain satisfied with an examination of one particular organ for all poisons that might come into question.

The systematic general procedure employed in testing for poisons often need not be carried through to completion as, for example, in a toxicologico-chemical examination where the search from the outset can be limited to a definite poison.

The custom is to deliver to the forensic chemist in separate containers all organs and animal fluids, such as parts of the stomach, intestines and contents, pieces of liver, kidney and spleen, and also blood and urine. Each part should be examined by itself, even when all the parts belong to the same case. The only exception to this rule is a case demanding the speediest information possible as to presence of poison in the parts of the given cadaver. In order to

answer such a question as quickly as possible, the author takes a good average sample of each part, organs as well as body-fluids, mixes them and examines the mixture for poison by the systematic general procedure. If this preliminary examination discloses a definite poison, it is sometimes, though not always, of interest to determine in what organs or body-fluids the given poison is located.

Before they are subjected to chemical examination for poison, organs such as liver, spleen, kidneys, heart, brain, or pieces of the stomach and intestines with contents should be brought to as fine a state of division as possible. For this purpose strong anatomical scissors that are sharp and thoroughly clean are very useful. Organs may be held by forceps or tongs made of German silver or nickel. A small meat-machine that has been carefully cleaned is also very convenient for this purpose.



CONTENTS

Author's Preface	PAGE v
Translator's Preface	vii
Introduction	ix

CHAPTER I

PHOSPHORUS AND OTHER POISONS VOLATILE FROM ACID SOLUTION

PHOSPHORUS	i
Scherer test, 1, Mitscherlich test, 3; Phosphorus sesquisulphide, 7; Dusart-Blondlot test, 8 (a) In Fresenius-Neubauer apparatus, 11; (b) In Hilger-Nattermann apparatus, 12, (c) Decomposition of silver phosphide (Stich), 14, Scherer and Dusart-Blondlot estimation of phosphorus by Fresenius-Neubauer method, 16; Detection and estimation by Nattermann-Hilger method, 18, Volatile phosphorus compounds in putrefaction and action of nascent hydrogen upon phosphorus compounds of animal body, 19, Stability in cadaver, Absorption and toxic action, 21, Metabolism in phosphorus poisoning, 22; Oxidation, phosphorescence, and conductivity of phosphorized air, 24, Detection of phosphorous acid in cadaver, Hypophosphites, 25	

FURTHER EXAMINATION OF DISTILLATE

HYDROCYANIC ACID	27
Schönbein preliminary test, 28; Distillation in carbon dioxide and quantitative estimation, 32, In presence of potassium ferrocyanide, Mercuric cyanide, Mercuric cyanide in presence of potassium ferrocyanide, 34, Formation normally from substances in body at higher temperature and by oxidation, 35, Relative stability in cadaver, 36, Hydrocyanic acid poisoning, symptoms and lethal dose; Absorption, 37; Post-mortem findings, 39	
PHENOLS	39
General behavior and reactions, 39, Supplement. Maltol, 42, Physiological behavior, 43.	
CARBOLIC ACID	43
Detection, 44, Quantitative estimations, Autenrieth-Beuttl gravimetric method, Beckurts-Koppeschaar volumetric method, 47, Messinger-Vortmann volumetric method, 49, Carbolie acid in presence of aniline, Toxic action and elimination, 50; Distribution in body in poisoning, 51.	

	PAGE
CRESOLS AND LYSOL	52
Examination of urine in lysol poisoning, 53	
THYMOL	54
Synthetic thymol, 54, Physiological behavior, 55, Detection, 56	
NAPHTHOLS	57
Preparation, 57, Medicinal use and toxic action of β -naphthol, Behavior of β -naphthol and naphthalene in organism; Detection, 58	
CHLOROFORM	59
Detection, 60, Estimation in cadaver; Quantitative estimation by Fujiwara's test, 62, Behavior in human organism, 63, Distribution in cadaver, 64	
CHLORAL HYDRATE	64
Preparation, 64, Behavior in animal body, Detection, 65, Estimation, 66, Archangelsky's estimation in blood and tissues, 67.	
BUTYL CHLORAL HYDRATE	67
IODOFORM	68
Detection, 68, Behavior in organism, Detection of iodine in urine in iodoform poisoning, 69	
NITROBENZENE	69
Toxic action, Behavior in cadaver, Detection, 70.	
ANILINE	71
Toxic action, 71, Aniline urine, Detection, 72	
ALCOHOL	73
Behavior and elimination in animal body, 73, Distribution in animal body in poisoning, Detection, 74	
ACETONE	76
Occurrence in urine; Detection, 76, Detection in urine, Detection in presence of alcohol, 77.	
ISOAMYL ALCOHOL	78
Toxic action; Detection, 78, In alcohol and brandy, 79.	
FORMALDEHYDE	79
Physiological action, 79, Detection, 80, Quantitative estimation 1. Romijn iodimetric method, 87, 2. Estimation by sodium sulphite (Lemme and Doby), 88.	
METHYL ALCOHOL	89
Physiological action, 90, Detection, 91. In cadavers (Juckenack), 94, In cadavers and urine by p-bromo-benzoyl chloride, 96.	
FORMIC ACID	97
Quantitative estimation, 98; In presence of salicylic acid, 99.	
BITTER ALMOND WATER AND BENZALDEHYDE	99
Detection of hydrocyanic acid in oil of bitter almonds.	
CARBON DISULPHIDE	100
Toxic action; Detection, 101, Estimation in air, 102	
PRESERVATION OF CADAVERIC MATERIAL FOR TOXICOLOGICAL EXAMINATION	103

CONTENTS

CHAPTER II

	P
ORGANIC SUBSTANCES NON-VOLATILE FROM ACID SOLUTION BUT EXTRACTED BY ACIDIFIED ALCOHOL	
A PREPARATION AND EXAMINATION OF ETHER EXTRACT OF AQUEOUS TARTARIC ACID SOLUTION	1
PICROTOXIN	
Properties, Toxic action, Detection, 112, Detection in beer and urine, 114	
COLCHICIN	1
Constitution, Toxic action, Detection, 116; See Appendix, 648	
PICRIC ACID	1
Preparation, Properties, toxic action, detection.	
ACETANILIDE	1
Preparation, properties, toxic action, reactions, Acetanilide urine, 125	
PHENACETINE	1
Preparation, 126, Physiological action, reactions, 127, Phenacetine urine, 129	
LACTOPHENINE	1
Preparation, physiological action, reactions.	
SALICYLIC ACID	1
Preparation, properties, physiological action, reactions, Detection in presence of phenols, 133, Estimation and detection in urine, 134	
SALOL	1
Preparation, physiological properties, Reactions, 136	
ASPIRINE	1
Preparation, physiological properties, detection	
VERONAL	1
Preparation, toxic action, 138, Detection, 139; In organs and duration of detection in cadaver, 140	
ANTIPYRINE	1
Preparation; Properties, physiological behavior, reactions, 142, Detection in urine (Stas-Otto), 143, Estimation, 144.	
CAFFEINE	1
Properties, synthetic caffeine, Physiological action, elimination, detection, 147.	
B EXAMINATION OF ETHER EXTRACT OF AQUEOUS ALKALINE SOLUTION	1
Preliminary tests for nitrogenous bases and purification of ether residue, 151, Experiments upon animals, putrefactive products of human organs, and Stas-Otto method, 152	
CONINE	15
Properties, constitution and synthesis, Toxic action, reactions, 154.	
NICOTINE	15
Properties, constitution and synthesis, Toxic action and detection, 158.	
ANILINE	16
For further information, see page 71.	
VERATRINE	16
Preparation, properties, toxic action, 161; Detection, 162.	

	PAGE
STRYCHENINE	164
Properties, constitution, 165, Toxic action, behavior in animal organ- ism and elimination, 166, Detection, 168, In presence of brucine, 170	
BRUCINE	171
Properties, constitution, Toxic action, detection, 172	
ATROPINE	173
Properties, constitution, 174, Physiological action, 175, Stability during cadaveric putrefaction and detection, 176	
HOMATROPINE	181
Detection	
SCOPOLAMINE	182
Properties, constitution, Detection, 184, Combination with morphine, 185, See Appendix, page 657	
COCAINE	185
Properties, constitution; Physiological action, elimination and detec- tion, 187, In presence of novocaine in cadaver, 190	
PHYSOSTIGMINE	191
Properties, Physiological properties, detection, 192	
PILOCARPINE	194
Properties, constitution; Physiological action, 195, Detection, 196	
CODEINE	197
Properties, preparation, constitution, physiological action, 198, Detec- tion, 199, Differentiation from morphine, 201	
NARCOTINE	201
Properties, constitution, 202, Physiological action, 203, Detection, 204	
HYDRASTINE	205
Preparation, properties, constitution, Physiological action, detection, 208	
QUININE	209
Properties; Constitution, 210, Physiological action, 212; Elimination from man, detection, 213.	
CAFFEINE	216
For further information, see page 145	
ANTIPYRINE	216
For further information, see page 141	
PYRAMIDONE	216
Preparation, properties, detection, Behavior in human body and elim- ination, 218	
C EXAMINATION OF ETHER EXTRACT AND CHLOROFORM EXTRACT OF SOLUTION ALKALINE WITH AMMONIA	218
α. ETHER EXTRACT	
APOMORPHINE	219
Preparation, properties, constitution; Detection, 221	
β CHLOROFORM EXTRACT	
Preliminary morphine test, 223; Purification of crude morphine, extrac- tion with ether containing alcohol, 224, Extraction with isobutyl alcohol, 225.	

CONTENTS

xvii

	PAGE
MORPHINE	225
Properties, constitution, Physiological action, 227, Elimination, stability during cadaveric putrefaction, 228, Detection, 230	
NARCEINE	234
Properties, constitution, Physiological action, detection, 235	
BEHAVIOR OF IMPORTANT ALKALOIDS TOWARD CERTAIN REAGENTS	237
SILICO-TUNGSTIC ACID AS ALKALOIDAL REAGENT	240

CHAPTER III

METALLIC POISONS

DESTRUCTION OF ORGANIC MATTER (Fresenius-v Babo)	242
Modification of Vintlesco, 245, By free chloric acid and by Mai method, 246	
EXAMINATION OF SOLUTION FOR METALLIC POISONS	246
Precipitation by hydrogen sulphide, Extraction of precipitate with ammonia-ammonium sulphide, 248	
HYDROGEN SULPHIDE PRECIPITATE EXAMINED FOR METALLIC POISONS I	249
Detection of arsenic 1 Berzelius-Marsh test, 250, Differences between arsenic and antimony spots and mirrors, 253, 2 Fresenius-v Babo test, 255, Detection of antimony, tin and copper, 256	
HYDROGEN SULPHIDE PRECIPITATE EXAMINED FOR METALLIC POISONS II	257
Mercury, 258, Lead, copper, bismuth and cadmium, 259	
EXAMINATION FOR CHROMIUM AND ZINC (METALLIC POISONS III)	260
EXAMINATION OF RESIDUE FOR BARIUM, LEAD AND SILVER (METALLIC POISONS IV)	262
TOXIC ACTION AND ELIMINATION OF METALLIC POISONS FROM HUMAN ORGANISM	264
ARSENIC	264
Absorption and toxic action, Distribution, deposition in human body, and lethal dose, 265, Deposition in hair, 267, Normal occurrence of arsenic (?), copper and zinc in human body, 268	
ANTIMONY	269
BARIUM	269
TOXIC ACTION OF HEAVY METALS	270
LEAD	271
CHROMIUM, COPPER	272
MERCURY	273
SILVER	274
URANIUM, BISMUTH	275
ZINC	276
TIN	277
SYSTEMATIC PROCEDURE IN CHEMICAL EXAMINATION FOR POISONS	278
Synopsis of Group I, Of Group II, 281, Of Group III, 288	

CHAPTER IV

EXAMINATION FOR POISONS NOT IN THREE MAIN GROUPS

	PAGE
A INORGANIC SUBSTANCES	290
MINERAL ACIDS	290
HYDROCHLORIC ACID	290
NITRIC ACID	291
SULPHURIC ACID	294
Corrosive action, detection	
HYDROFLUORIC ACID AND SOLUBLE FLUORIDES	295
Official directions for detection of hydrofluoric acid and salts, 296	
BROMINE AND ALKALI BROMIDES	296
Behavior of alkali bromides in human organism, detection and estimation of bromine in bromides, 297, Ashing of urine and colorimetric estimation of bromine in ash (Autenrieth), 298	
IODINE AND IODIDES	300
Physiological action, Detection of free iodine and estimation of iodine in urine, blood and organs after administration of alkali iodides, 301, Estimation of iodine in thyroid gland and in urine, 303	
HYDROGEN SULPHIDE	304
Toxic action when inhaled and detection in cadaver, In urine, in air and in water, 305, Estimation in air, 307, Colorimetric estimation of minimal quantities in air and water (Autenrieth-Brehl), 658.	
SULPHUR DIOXIDE AND SULPHEOUS ACID	307
Toxic action; Detection, estimation in air, 308, In cadaver, official directions for detection and estimation of sulphurous acid and salts and of hyposulphites in meat, 309	
BORIC ACID	310
Physiological action and elimination, Detection in urine, 311; Estimation 1. Jørgensen, 2 Partheil and Rose, 312; Official directions for detection of boric acid and salts in meat, 314.	
CHLORIC ACID AND CHLORATES	315
Toxic action, detection; Estimation, 316; Behavior during putrefaction, official directions for detection of chlorates in meat, 318	
OXALIC ACID	318
Toxic action, distribution in organs in oxalic acid poisoning, 319, Detection and estimation, 320.	
NITROUS GASES AND NITRITES	321
Toxic action; Detection, 322.	
DETECTION OF FREE ALKALIES	322
Corrosive action, 323.	
AMMONIA	323
FIXED ALKALIES	323
CARBON MONOXIDE	324
I. DETECTION AND ESTIMATION IN BLOOD: Toxic action; Properties of carbon monoxide blood A. Chemical detection, 325, B. Spectroscopic detection, 327, C. Approximately quantitative estimation of carboxy-haemoglobin in blood, 328.	

CONTENTS

xix

	PAGE
II DETECTION AND ESTIMATION IN AIR 1 Detection with blood, 2	
Detection with palladous chloride, Estimation (Gadamer), 330	
B ORGANIC SUBSTANCES	331
ORGANIC ARSENICAL COMPOUNDS	
CACODYLIC ACID	332
Preparation, properties, Detection in urine and organs, 333	
METHYL-ARSINIC ACID AND ARRHENAL	334
Preparation, properties, Estimation of arsenic in cacodylic acid and arrhenal, 335	
ATOXYL	336
Preparation, properties, use, detection, Elimination in urine, 339	
ACETYL-P-ARSANILIC ACID AND ARSACETINE	339
Estimation of arsenic in atoxyl and arsacetine (Rupp and Lehmann), 340	
SALVARSAN	340
Preparation, properties, Fate in human body, 342, Estimation of arsenic in salvarsan, 344	
NEO-SALVARSAN AND SODIUM-SALVARSAN	345
Preparation, properties, dose, Colorimetric estimation in urine (Autenrieth-Taegge), 346	
CANTHARIDIN	348
Properties, constitution, Toxic action, elimination, detection in organs, blood and urine, 349	
SYNTHETIC HYPNOTICS AND SEDATIVES	
General remarks upon hypnotics and sedatives	350
PROFONAL	351
Properties, physiological properties, 352	
LUMINAL	352
Preparation, properties, action, Detection, 353	
MEDINAL OR SODIUM-VERONAL	354
Beginning of action.	
SODIUM-LUMINAL	354
DIAL	354
Preparation, physiological action, Didial, 355	
ORGANIC HYPNOTICS CONTAINING BROMINE	
ADALINE	355
Preparation, properties, physiological action	
BROMURAL	357
Preparation, properties, physiological action	
NEURONAL	358
Preparation, properties; Detection, distribution of adalinc, bromural and neuronal in organism, 359	
DIOGENAL	360
Preparation, properties, pharmacological action	

	PAGE
NIRVANOL .	361
Preparation, detection, medicinal use, toxic action, 362.	
SULPHONAL	363
Preparation, properties, Detection, physiological action, 364; Detection in urine, detection of haematoporphyrin in urine in sulphonal intoxication, 365	
TRIONAL	366
SANTONIN .	366
Properties, toxic action, behavior in animal body, detection, Constitution, examination for santonin, sulphonal, trional, 369	
CERTAIN ALKALOIDS OF RARE OCCURRENCE	
ACONITINE	370
Properties, constitution, reactions, Physiological behavior, 371	
PSEUDO-ACONITINE	371
Biological detection of aconitine (Fühner), 372	
ARECOLINE	373
Properties, physiological action, use; Constitution, synthesis, 374; Detection, 376.	
ALKALOIDS OF POMEGRANATE BARK	376
Isolation, 377.	
PELLETIERINE .	377
PSEUDO-PELLETIERINE	378
Therapeutic use, toxic action and detection, 379	
ALKALOIDS OF IPECACUANHA .	380
Isolation (Hesse), 381.	
EMETINE .	381
CEPHAELINE .	382
PSYCHOTRINE	383
Emetine and cephaeline Detection, constitution, Physiological action and medicinal use, 385.	
JOHIMBINE	386
COCAINE SUBSTITUTES	
NOVOCAINE	387
Properties, detection, Physiological action, 389	
ORTHOFORM (new)	389
STOVAINE	390
TUTOCAINE	391
PSICAINE	392
SUPRARENINE OR ADRENALINE	393
Properties, synthetic adrenaline; Detection, 394, Colorimetric estimation, 395	
TROPACOCAINE.	396
Reactions, 397, Physiological properties, 398.	
LOBELINE	398
Crystallized lobeline (Wieland), 399; Behavior toward reagents, 400, See Lobelia alkaloids, 655.	

CONTENTS

xxi

	PAGE
MUSCARINE	400
Properties, physiological action, 401, Elimination and detection, 402, Synthetic muscarines, 403.	
DERIVATIVES OF MORPHINE	
DIONINE	405
HEROINE	405
OXYDIMORPHINE	406
PAPAVERINE	408
Properties, constitution, detection, Physiological action, 410	
THEBAINE	410
THEOBROMINE	412
Properties, synthetic theobromine, Physiological properties, detection, 414	
SODIUM-THEOBROMINE-SODIUM SALICYLATE, OR DIURETINE	415
Estimation of theobromine-content	
THEOPHYLLINE	415
TOXALBUMINS	416
ABRIN	417
RICIN	417
CROTIN	418
CYTISINE	419
Properties, toxic action, detection.	
GLUCOSIDES AND SAPONIN SUBSTANCES	
DIGITALIS GLUCOSIDES	420
DIGITOXIN	420
Properties, constitution; Reactions, 423	
DIGITALIN	423
Properties, hydrolytic cleavage, Reactions, 424	
Detection of digitoxin in organs and urine (Cloetta-Fischer), 424.	
Behavior of digitoxin in organism, 425	
Physiological test for digitalis and other digitalis-like substances, 426.	
DIGITONIN	427
Properties, digitonin-cholesteride, Chemistry, 428	
STROPHANTHIN	431
Preparation, chemistry, detection; Colorimetric estimation (Auten- rieth-Quantmeyer), 433; Colorimetric estimation of strophanthin- content of tincture of Strophanthus, 435	
TOAD POISON	435
Color reactions, constitution, 436, Physiological action, 437	
SAPONINS OR SAPONIN SUBSTANCES	437
Properties, Occurrence, 438; Reactions in common and detection, 440; Physiological action, 441	
Detection in effervescing beverages (Brunner-Rühle), 443, Detection in oil-emulsions, 444	
Detection in beverages (Müller-Hüssly), 444	
Detection in presence of glycyrrhizin, 445; By haemolysis in Seltzer	

	PAGE
water, beer and wine, In flour and bread, 446	
Detection (Rosenthaler), 446	
Detection of corn-cockle in flour, 447	
Estimation in plant powders (Korsakow), 448	
SOLANINE AND SOLANIDINE	448
Occurrence, properties, Toxic action, detection, 449, Estimation in potatoes 1 Schmiedeberg-Meyer, 450, 2 v Morgenstern, 451	
EXAMINATION OF NATURAL PRODUCTS	
ERGOT	451
Constituents, 452, Reactions of ergotoxine and ergotinine, 453, Detection of schlererythrin (E Schaer), of ergot in flour, bread, of choline in ergot, 454, Detection of alkaloids, ergotinine and ergotoxine, and estimation in ergot (Keller), Tenosine Bayer, 455	
OPIMUM	455
Meconic acid Properties, constitution, detection, 456	
Meconine Properties, constitution, detection	
PTOMAINES	457
Isolation (Brieger), 458, I Ptomaines from human cadaver, 459, II Ptomaines from horse-flesh, 460; Separation of cadaverine and putrescine (Baumann-v Udránszky), 461, Ptomaines resembling alkaloids, 462	

CHAPTER V

APPARATUS, SPECIAL METHODS OF EXAMINATION, AND SPECIAL EXAMINATIONS
CERTAIN APPARATUS

EXTRACTORS	465
AUTENRIETH-KOENIGSBERGER WEDGE-COLORIMETER	468
MICRO-SUBLIMATION OF ALKALOIDS IN VACUO (Eder)	471
A. EXAMINATIONS IN FIELD OF INORGANIC CHEMISTRY	
ELECTROSCOPIC DETECTION OF MINUTE QUANTITIES OF PHOSPHORUS (Schenk and Scharff)	476
DETECTION OF FREE PHOSPHORUS IN PHOSPHORUS SESQUISULPHIDE (Schroeder)	479
DETECTION OF TRACES OF PHOSPHORUS IN LARGE QUANTITIES OF PHOSPHORUS SESQUISULPHIDE (Aronstein)	480
DETECTION OF PHOSPHORUS IN PHOSPHORATED OILS.	480
1 Straub, 480, 2 Mitscherlich test, 481.	
QUANTITATIVE ESTIMATION OF PHOSPHORUS IN PHOSPHORATED OIL AND PHOSPHORATED COD-LIVER OIL	481
1 Enell, 481, 2 Katz, 483; 3 Fränkel-Stich, 484	
DETECTION AND ESTIMATION OF SMALL QUANTITIES OF ARSENIC	
DESTRUCTION OF ORGANIC MATTER AND DETECTION OF MINUTE QUANTITIES OF ARSENIC (Lockemann)	486
Method of destruction, For urine, blood, parts of organs and meat, 487; Separation of arsenic, 488, Detection of arsenic, 489, Activators and arsenic-free chemicals, 491; Purification of potassium and sodium nitrates, 492.	

	PAGE
DETECTION OF ARSENIC BY ASHING METHOD (Strzyzowski)	493
Marsh-Strzyzowski apparatus for detection of arsenic, 494	
GUTZEIT TEST FOR ARSENIC	496
Modifications of Flückiger, Smith, Beck-Merres, 497, Quantitative estimation of arsenic and antimony (Sanger), 499	
BETTENDORFF TEST FOR ARSENIC	501
Preparation and sensitiveness of reagent, 502	
REINSCH TEST FOR ARSENIC AND SPECIAL USE	504
Modified for detection of arsenic in beer (Chapman and Allen)	
BIOLOGICAL TEST FOR ARSENIC BY MEANS OF MOULD	505
DETECTION AND ESTIMATION OF ARSENIC (Schneider-Fyfe-Beckurts)	509
DESTRUCTION OF ORGANIC MATTER BY PERMANGANATE OR PERSULPHATE AND IODIMETRIC ESTIMATION OF ARSENIC (Rupp-Lehmann)	512
COLORIMETRIC ESTIMATION OF MINUTE QUANTITIES OF ARSENIC IN CADAVERS (Fühner)	514
DETECTION AND ESTIMATION OF ARSENIC (Engel and Bernard)	517
ELECTROLYTIC DETECTION OF ARSENIC	518
ELECTROLYTIC ESTIMATION OF MINUTE QUANTITIES OF ARSENIC (Mai and Hurt)	519
Examination of cadavers and other organic material, 521, Procedure with Frenchs-Rodenberg apparatus, 522	
COLORIMETRIC ESTIMATION OF MINUTE QUANTITIES OF ARSENIC (Autenrieth-Breh)	524
LEAD	526
Detection of small quantities of lead in urine, 526	
MERCURY	528
I QUALITATIVE DETECTION IN URINE Bardach, Glaser and Isenburg, Salkowski, 529, Jolles method modified by Oppenheim, 530, Peiselstein and Abelin, 531	
II QUANTITATIVE ESTIMATION IN URINE Schuhmacher and Jung, 531, Farup method modified by Beckers, 533, Autenrieth-Montigny colorimetric method, 534	
III ESTIMATION IN CORROSIVE SUBLIMATE TABLETS AND IN OFFICIAL MERCURIAL OINTMENTS 1 Corrosive sublimate tablets, 537, 2 White precipitate ointment, 538, 3 Gray mercurial ointment and mercuric oxide ointment, 538	
ESTIMATION OF SILVER IN ORGANS AND URINE	540
B EXAMINATIONS IN FIELD OF ORGANIC CHEMISTRY	
HYDROCYANIC ACID	540
1 Colorimetric estimation of minute quantities (Autenrieth-Quantmeyer), 2 Estimation of total hydrocyanic acid in Bitter Almond Water (German Pharmacopoeia), 542	
DETECTION OF METHYL ALCOHOL IN BRANDY AND IN OTHER ALCOHOLIC LIQUIDS	543
1. Oxidation to formaldehyde and detection of latter (a) Fendler and Mannich, (b) Wirthle, 544	

	PAGE
2 Oxidation to formaldehyde and condensation of latter with dimethyl-aniline (Trillat and Wolff), 545, Detection with caramel or sugar, 546	
3. Detection in presence of ethyl alcohol (Denigès), 546	
DETECTION OF FORMALDEHYDE IN FOODS AND CONDIMENTS	546
1 Detection in meat (German Official Directions), 2 In milk and other foods (modified Hehner's test) 547, 3 By means of fuchsine-sulphurous acid-hydrochloric acid (Fincke), 548	
Colorimetric estimation (Autenrieth), 548, In urine, 550	
Estimation in trioxymethylene tablets (Blank and Finkenbeiner), 551	
DETECTION AND ESTIMATION OF QUININE IN URINE	552
(a) Detection (Giemsa and Halberkann); (b) Estimation (Meyer).	
ESTIMATION OF QUININE AND STRYCHNINE TOGETHER (Harrison and Gair)	554
ESTIMATION OF STRYCHNINE WITH QUININE (Simmonds)	555
EXAMINATION OF CHLORAL URINE	555
Isolation of urochloralic acid (a) v Mering, (b) Külz, 556.	
EXAMINATION OF CARBOLIC ACID URINE	557
1 Estimation of volatile phenols (a) Mooser, (b) Ellinger-Hensel, 558	
2 Estimation of sulphuric acid (total, sulphate and ethereal) in urine (a) Total, 559, (b) Ethereal (Baumann), 560, Salkowski, 561; (c) Sulphate	
DETECTION OF MORPHINE IN ORGANS	561
1 Cloetta, 561; 2 Marquis, 562	
COLORIMETRIC ESTIMATION OF MORPHINE	563
In urine, 564	
EXAMINATION OF PYRAMIDONE URINE (Jaffé)	566
Detection of antipyril-urea and rubazonic acid	
DETECTION AND ESTIMATION OF VERONAL IN URINE	567
DETECTION OF SALICYLIC ACID IN FOODS AND BEVERAGES	569
1. In wine (German official directions), 2 In milk, In meat and meat products, 570	
TOXICITY OF CHEMICAL COMPOUNDS ESTIMATED BY BLOOD HAEMOLYSIS (Vandevelde)	570
Physiological salt solution and haemolysis, 572	
AQUEOUS CHLORAL HYDRATE SOLUTIONS IN TOXICOLOGICAL ANALYSIS (Mauch)	572
PHOSPHORUS IN INFLAMMABLES	574
German official directions for their examination	
LEGAL PROVISIONS, METHODS OF DETECTING ARSENIC AND TIN IN COLORED FOODSTUFFS, AND ARSENIC IN SPUN OR WOVEN GOODS	577

CHAPTER VI

QUANTITATIVE ESTIMATION OF ALKALOIDS AND OTHER PRINCIPLES IN CRUDE MATERIALS AND THEIR PREPARATIONS

ALKALOIDS AND ESTIMATION IN DRUGS AND PREPARATIONS (German Pharmacopoeia)	583
ALKALIMETRIC ESTIMATION OF ALKALOIDS (German Pharmacopoeia)	584

	PAGE
ALKALOIDS BY PICROLONATE METHOD (Matthes and Rammstedt)	585
Estimation of morphine, codeine and stypticine in solutions, tablets and sugar triturates, 586	
ALKALOIDS BY POTASSIUM BISMUTHOUS IODIDE; DECOMPOSITION OF PRECIPITATE BY ALKALI HYDROXIDE-CARBONATE (Thoms)	587
ALKALOID IN IPECACUANHA AND PREPARATIONS	589
1 Radix Ipecacuanhae, 2 Tinctura Ipecacuanhae	
CANTHARIDIN IN SPANISH FLIES	590
ALKALOIDS IN CINCHONA BARK AND PREPARATIONS	591
1. Cinchona bark, 2 Aqueous and alcoholic cinchona extract, 594, 3 Fluid extract of cinchona, 595; 4. Tincture of cinchona and compound tincture of cinchona, 596	
ALKALOID IN POMEGRANATE BARK	596
HYDRASTINE IN HYDRASTIS ROOT AND FLUID EXTRACT	597
1 Hydrastis root, 2 Fluid extract of hydrastis, 598	
HYOSCYAMINE IN BELLADONNA LEAF, HYOSCYAMUS LEAF, AND OFFICIAL EXTRACTS	598
1 Belladonna and hyoscyamus leaves, 2 Extract of belladonna and of hyoscyamus, 599	
MORPHINE IN OPIUM AND PREPARATIONS	600
1 Powdered opium, 2 Extract of opium, 3 Simple tincture of opium, 601, Morphine meconate, 602	
STRYCHNINE AND BRUCINE IN NUX VOMICA AND PREPARATIONS	602
1 Nux vomica, 2 Extract of nux vomica, 3 Tincture of nux vomica, 603.	
ASSAYING OF CRUDE MATERIALS AND PREPARATIONS NOT IN GERMAN PHARMACOPOEIA	604
QUININE IN CINCHONA BARK AND PREPARATIONS BY SULPHATE METHOD (Carles)	604
1 Cinchona bark; 2 Extract of cinchona, 605	
COLCHICIN IN COLCHICUM SEEDS AND CORMS (Katz and Bredemann)	606
HYDRASTINE IN HYDRASTIS ROOT AND EXTRACT BY PICROLONATE METHOD (Matthes and Rammstedt)	606
1 Fluid extract; 2. Hydrastis root; 3 Estimation of berberine, 607	
CAFFEINE IN COFFEE, TEA, COLA NUTS, AND GUARANA PASTE	607
1 Keller's method; 2 Hilger-Juckenack method, 608; 3. Trillich-Göckel modification of Hilger's method, 4 Trillich-Göckel modification of Socolof's method, 609, 5 Katz's method; 6 Dietrich's procedure for estimating total alkaloids in cola nuts, 610	
NICOTINE IN TOBACCO AND EXTRACTS	611
1. Kissling's method, 2 Keller's method, 3 Chapin's silico-tungstic acid method, 612, 4 Rasmussen's method for tobacco and extracts, 613, Physiological action of tobacco smoke, 614	
PILOCARPINE IN JABORANDUM LEAVES	615
1 Fromme's method; 2 Matthes-Rammstedt method	
PIPERINE IN PEPPER	615
1 Koenig's method, Cazeneuve-Caillet method, 617.	

	PAGE
SANTONIN IN WORMSEED	617
1 Thaeter's method, 2 Katz's method, 618 In santonin troches and santonin chocolate troches, 619	
STROPHANTHIN IN STROPHANTHUS SEEDS AND TINCTURE (Fromme)	619
1 Strophanthus seeds, 2 Tincture of strophanthus Fromme's method modified by Bodinus, 620	
ALKALOID IN NUX VOMICA	621
1 Keller's method, 2 Picrolonate method (Matthes-Rammstedt) (a) Extract of nux vomica, (b) Tincture of nux vomica, (c) Nux vomica seeds, 622, 3 Azadian's method, 623	
STRYCHNINE WITH BRUCINE	623
Keller's method modified by Gordin	
THEOBROMINE AND CAFFEINE IN CACAO AND CHOCOLATE	624
Beckurts-Fromme method modified by Prochnow Separation of theobromine from caffeine	
BIOLOGICAL VALUE OF DIGITALIS LEAVES AND PREPARATIONS	625
CONSTITUENTS OF MALE FERN AND EXTRACT, FILICIN IN LATTER	626
MILTA-CRESOL IN CRUDE CRESOL	629
CRESOL IN CRESOL SOAP SOLUTION	631

SUPPLEMENT

DETECTION OF BLOOD STAINS AND HUMAN BLOOD

1 DETECTION OF BLOOD STAINS	631
Haematin, Spectroscopic detection of blood, 633, Schönbein-van Deen test, 634, Vitali's procedure, Schaer's procedure, Schaer's alone test, 635, Adler's blood tests with leuco-malachite green and benzidine, 636	
2 BIOLOGICAL DETECTION OF HUMAN BLOOD	637

PREPARATION OF REAGENTS

A GENERAL ALKALOIDAL REAGENTS	638
B SPECIAL ALKALOIDAL AND OTHER REAGENTS	641
C THE INDICATOR IODEOSINE	644
D CURCURMINI AND CURCURMINE PAPER	644

APPENDIX

NARCOSIS THEORY (Meyer and Overton)	646
CONSTITUTION OF COCHICIN AND COCHICEIN	648
ALKALOIDS OF LOBELIA INFLATA	655
Properties; Pharmacological action, 656	
SCOPOLAMINE, SCOPINE AND SCOPOLINE	657
HYDROGEN SULPHIDE IN WATER AND AIR COLORIMETRICALLY ESTIMATED (Autenrieth-Breh)	658

INDEX OF NAMES	661
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INDEX OF SUBJECTS	675
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DETECTION OF POISONS

CHAPTER I

VOLATILE POISONS

Phosphorus and Other Poisons Volatile with Steam from Acid Solution

Many materials acidified with tartaric or dilute sulphuric acid and distilled with steam yield distillates containing those substances that are volatile under these conditions. As far as toxicological investigations are concerned, the most important compounds of this class are:

Phosphorus,	Chloroform,	Ethyl Alcohol,
Hydrocyanic Acid,	Chloral Hydrate,	Methyl Alcohol,
Carbolic Acid,	Butyl Chloral Hydrate,	Amyl Alcohol,
Cresols,	Iodoform,	Formaldehyde,
Thymol,	Nitrobenzene,	Acetone,
Naphthols,	Aniline,	Formic Acid.
	Carbon Disulphide,	

PHOSPHORUS

Scherer's¹ Preliminary Test for Phosphorus.—This test should always be made before testing for yellow phosphorus by distilling with steam. The principle of this test is that yellow phosphorus is volatile on gentle warming, or even at room temperature. Its vapor will blacken silver nitrate either from formation of silver phosphide, or because phosphorous acid produced by oxidation of phosphorus reduces silver from silver nitrate. This test, however,

¹ J. Scherer. Detection and Estimation of Phosphorus and Phosphorous Acid in Poisoning Cases. Ann. d. Chem. 112 (1859), 214

may lead to error, for the material may give off hydrogen sulphide which will also blacken silver nitrate paper.

To establish the presence or absence of hydrogen sulphide, the vapor given off should be tested with "lead paper." Hydrogen sulphide will blacken this paper but phosphorus will not. Addition of cadmium sulphate to the material will remove hydrogen sulphide but have no effect upon phosphorus.

Procedure.—Place the finely divided material in a small flask and cover with water, if a sufficient quantity is not present. Cut a V-shaped slit in the cork and place the latter loosely in the mouth of the flask so that the two strips of filter paper hang free (Fig 1).



FIG 1

Moisten one strip with silver nitrate and the other with lead acetate solution¹ Warm gently upon the water-bath (40–50°)² and allow to stand for some time protected from light. If the silver but not the lead paper is blackened, yellow phosphorus may be present. If both papers are blackened, hydrogen sulphide is also present. In the latter case, yellow phosphorus may be present with hydrogen sulphide. In absence of hydrogen sulphide, blackening of the silver paper is not final proof of yellow phosphorus, for any volatile organic substance having reducing properties, such as formaldehyde (H CHO), or formic acid (H COOH), may give the same result.

Scherer's test is of value in proving absence rather than presence of yellow phosphorus. It is a good preliminary test, as it excludes phosphorus if the silver paper is unchanged

Distillation

Place a portion of finely divided and thoroughly mixed material in a large round-bottom flask and add enough distilled water for free distillation. Then add tartaric acid solution drop by drop until the mixture is acid after thorough shaking. In examining animal material, such as the stomach or intestines and contents, or

¹ To prepare a sensitive "lead paper," use an alkaline lead oxide solution instead of lead acetate. This is obtained by mixing a solution of a lead salt with excess of sodium hydroxide solution.

² Temperatures in this book are expressed in Centigrade degrees Tr.

organs, such as liver, spleen, kidneys, or brain, it is often unnecessary to add much water because usually enough is present. First chop the material in a wooden tray with a steel knife. In a medico-legal case the tray should be new. A meat-machine that has been carefully cleaned may be used. Thin the material with a little distilled water, acidify with dilute tartaric or sulphuric acid and finally distil.

If Scherer's test is positive, begin distilling with the Mitscherlich apparatus, but if negative, distil in the usual way with a Liebig condenser.

Mitscherlich¹ Method of Detecting Phosphorus

The principle of this method is that yellow phosphorus volatilizes with steam and becomes luminous in contact with air. Phosphorescence is best seen in a dark room.

Procedure.—Arrange the apparatus as shown in Fig. 2. Support the condenser in a vertical position and connect the upper end with the flask by a glass tube about 8 mm internal diameter. This tube has two right-angle bends and each end passes through a cork. Have condenser and tube scrupulously clean to avoid interference with phosphorescence.

Have the flask at most not more than a third full. This precaution is necessary because many materials, containing protein substances, such as albumin, albumose, etc., and starchy matter, when distilled in aqueous solution, cause more or less foaming that may carry over solid matter into the receiver. Use as the receiver an Erlenmeyer flask, containing a little distilled water (3-5 cc.), into which the end of the condenser dips. This precaution prevents loss of easily volatile substances such as hydrocyanic acid, chloroform and alcohol. Heat the flask upon a wire gauze of fine mesh, asbestos plate, or sand-bath, and bring the contents to boiling by raising the temperature gradually. There is some danger of burning or carbonizing organic matter on the bottom of the flask, if heat is applied too strongly or rapidly. When boiling begins, make the room as dark as possible and watch for phosphorescence in the tube and condenser. It usually appears as a luminous ring or band in the upper part of the condenser. When this is distinctly visible, presence of yellow phosphorus is established. Phosphorescence

¹ E. Mitscherlich, *Method of Detecting Phosphorus in Poisoning Cases*, *Journ. f. prakt. Chem.* 66 (1855), 238.

during distillation with steam is very characteristic of yellow phosphorus and frequently is the only positive test for this element¹ In absence of interfering substances, the delicacy of the Mitscherlich test is very great. Hilger found that 0.06–0.3 mg. of phosphorus in

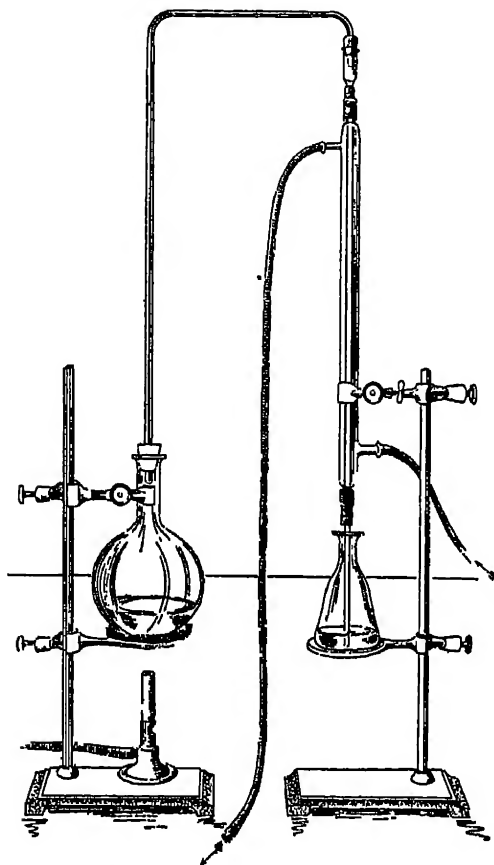


FIG. 2.—Mitscherlich apparatus

200 cc. of water could be distinctly recognized and Fresenius observed that phosphorescence from 1 mg. of phosphorus lasted

¹ During distillation in the Mitscherlich apparatus, phosphorus sesquisulphide, P_4S_6 , gives rise to phosphorescence like that produced by yellow phosphorus. If this phosphorus compound is present in the material, yellow phosphorus can be detected only with the electroscope by the method of Schenck and Scharff described in Chapter V (see page 476).

for half an hour. Obviously duration of phosphorescence depends upon rapidity of distillation.

Phosphorescence is a process of oxidation by which phosphorus vapor is changed to phosphorous acid. Should it not appear immediately, distillation must be continued for some time, since certain substances such as ethyl or methyl alcohol, ether, turpentine, acetic ether, and benzine, as well as many ethereal oils either prevent phosphorescence entirely or seriously retard it. Carbolic acid, creosote, chloroform, chloral hydrate, as well as hydrogen sulphide, may completely prevent phosphorescence. When yellow phosphorus is present, phosphorescence may also be prevented by substances that can convert phosphorus into non-volatile compounds. All oxidizing agents, and particularly salts of mercury,¹ copper and silver, are capable of acting in this manner. By this action, phosphorus is converted into oxygen or metallic compounds. Mercuric chloride and other mercury compounds can entirely prevent phosphorescence. Possibly mercuric chloride carried over by steam is reduced to metallic mercury by phosphorus vapor. In that case the metal should appear in the distillate. The fact that both metallic mercury and phosphoric acid can be detected in the distillate favors the supposition that action takes place between phosphorus vapor and mercuric chloride. In presence of those volatile substances that do not prevent volatilization of phosphorus but do interfere with the appearance of phosphorescence, the latter often will appear, though not always, when these interfering substances have been removed by distillation, provided their quantity is not too large and that of phosphorus too small. Distillation in such cases should be of long duration. By a modification of the Mitscherlich apparatus, Habermann and Oesterreicher were able to observe phosphorescence even in presence of ethyl alcohol. Where the steam entered the condenser they introduced a dropping-funnel containing cold water. The instant steam reached the condenser they injected a spray of water. Other substances that vaporize, such as turpentine and volatile phenols, permanently prevent appearance of phosphorescence. This is an important matter in view of the fact that resinified turpentine is used as an antidote for phosphorus poisoning and volatile phenols appear in the putrefaction

¹ K. Polstorff and J. Mensching. Test for Phosphorus by the Mitscherlich Method in Presence of Chlorides of Mercury. *Ber d Deutsch chem. Ges.* 19 (1886), 1763.

of proteins In presence of elementary phosphorus the distillate has the odor of this element and sometimes contains globules of phosphorus together with phosphorous acid.

In any event, even when phosphorescence has not been observed, convert phosphorus that may be present as well as phosphorous acid into phosphoric acid This may be done by evaporating a considerable portion of the distillate in a porcelain dish upon the water-bath in presence of an excess of saturated chlorine water, strong bromine water, or a little fuming nitric acid. Dissolve the residue in a little water, divide the solution into two equal portions, and make the two following tests for phosphoric acid:

1. **Ammonium Molybdate Test.**—Acidify the solution with a few drops of concentrated nitric acid Add an equal volume of ammonium molybdate solution and warm to about 40° Phosphoric acid precipitates yellow ammonium phospho-molybdate.

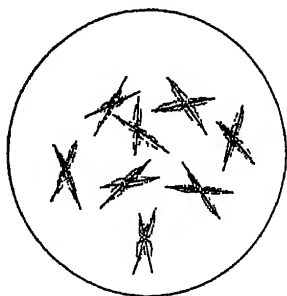


FIG. 3 —Ammonium magnesium phosphate crystals

2. **Ammonium Magnesium Phosphate Test.**—Add magnesia mixture¹ to the second portion. Phosphoric acid gives a white crystalline precipitate of ammonium magnesium phosphate, $(\text{H}_4\text{N})\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$.

Vigorous shaking favors precipitation. When only traces of phosphoric acid are present, long standing is necessary before the precipitate appears Always examine the precipitate with the microscope. It should consist of well-formed crystals or at least be crystalline. These crystals are transparent, acicular prisms (Fig. 3).

Notes upon Detection of Phosphorus by Mitscherlich Method

The author regards it of the utmost importance to test for phosphoric acid in the distillate following oxidation in the manner described, especially when yellow phosphorus, owing to the defects of the Mitscherlich method mentioned above, cannot be recognized by the eye When the distillate prepared as directed gives a positive test for phosphoric acid, the material contains yellow phosphorus,

¹ Magnesia mixture is a clear solution prepared by mixing equal volumes of magnesium chloride, ammonium chloride and ammonium hydroxide (about 10 per cent.) solutions It contains the readily soluble double chloride of ammonium and magnesium which is not decomposed by ammonium hydroxide. This reagent is prepared as needed and should be perfectly clear and colorless.

provided phosphorus sesquisulphide is not present. When phosphorescence is not observed, the distillate obtained by the Mitscherlich method may also be shaken with silver nitrate solution. If a black precipitate appears, it can be filtered off at once, washed and examined for phosphorus in the Dusart-Blondlot apparatus (see page 8).

In all cases the author does not recommend testing the Mitscherlich distillate for phosphorous acid with silver nitrate and mercuric chloride, for distillates from cadaveric material quite often contain such substances as hydrogen sulphide, aldehyde-like bodies, or formic acid (a normal constituent of human urine). They give the same reactions with these two reagents that phosphorous acid does and consequently can simulate the presence of the latter. Slightly characteristic reactions of this description in the hands of not over-critical observers lead only to harm. At the same time such tests are merely a waste of expensive material.

The smallest quantity of yellow phosphorus that can be detected with certainty by the Mitscherlich method is 0.06 mg. When 200 cc of water containing 0.3 mg of phosphorus were distilled, there was brilliant phosphorescence for 5 minutes (Nattermann and Hilger). The degree of dilution seems to have no effect upon the result, at least not within limits occurring in practice. This also applies to hydrogen sulphide which is always given off when cadaveric material that has begun to putrefy is acidified and heated. When an acid aqueous solution is distilled in the Mitscherlich apparatus, the flask residue always contains phosphoric (H_3PO_4), phosphorous (H_3PO_3), and hypophosphorous (H_3PO_2) acids and red phosphorus. Distillation of a solution of 0.0644 gram of phosphorus gave only 71.33 per cent in the distillate. The residue contained—

Phosphorus as phosphoric acid (H_3PO_4)	18.93 per cent
Phosphorus as phosphorous acid (H_3PO_3)	2.15 per cent.
Phosphorus as hypophosphorous acid (H_3PO_2)	4.27 per cent
Phosphorus as red phosphorus	2.08 per cent.
	— — —
	28.33 per cent

A. Fischer¹ states that substances interfering more or less with the detection of phosphorus by the Mitscherlich method are usually less troublesome if Hilger and Nattermann's procedure is adopted (see page 18). The essential feature of this procedure consists in allowing steam charged with phosphorus to pass into the air from time to time, or in admitting air into the apparatus.

Phosphorus Sesquisulphide, P_4S_3 .—In the manufacture of matches that will light anywhere, this compound is recommended as a substitute for white phosphorus which is forbidden in Germany. When distilled from an acid aqueous solution, it produces the same phosphorescence exhibited by toxic yellow phosphorus. For this reason the Mitscherlich method of detecting phosphorus cannot be regarded as free from objection, unless absence of phosphorus sesquisulphide has been positively established.

In the examination of the distillate, obtained by distilling a given material in a current of carbon dioxide according to the Scherer-Fresenius-Neubauer method,

¹ A. Fischer. Contributions to the Detection of Phosphorus. Pfüger's Arch. 97 (1903), 578.

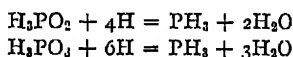
phosphorus sesquisulphide also interferes with the detection of phosphorus by the Dusart-Blondlot method, since in this case it is capable of simulating toxic elementary phosphorus. The physical method of R. Schenck and Scharff¹ is the only means of determining whether the given material contains elementary phosphorus or phosphorus sesquisulphide. Phosphorus vapor, probably because it undergoes oxidation even at ordinary temperature to phosphorus trioxide (P_2O_3), renders air a conductor of electricity, since it ionizes air and consequently discharges a charged electroscope. On the other hand, pure phosphorus sesquisulphide has no action whatever upon an electroscope at temperatures up to 50° and no considerable action up to 75° . Presence of only fractions of a milligram of white phosphorus renders air a conductor, for example, 0.004 mg. exerts a notable influence upon an electroscope. Temperatures of $35-55^\circ$ are best adapted for these experiments.

Whether there is an actual difference between the physiological, that is, the toxic action of elementary white phosphorus and that of phosphorus sesquisulphide appears to be unknown. The work of Morner, as well as that of Santeson and Malmgren, shows that a single dose of 0.5-1.0 gram of pure phosphorus sesquisulphide is apparently harmless. But if fed to animals for a longer time, it causes marked fatty degeneration of the liver as in phosphorus poisoning.

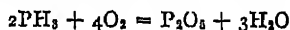
Detection of Phosphorus and Phosphorous Acid

(Dusart² and Blondlot³)

Elementary phosphorus and its lower oxidation products, hypophosphorous acid (H_3PO_2) and phosphorous acid (H_3PO_3) are reduced to gaseous phosphine (PH_3) by nascent hydrogen.



Under the same conditions, ordinary or ortho-phosphoric acid (H_3PO_4) and its anhydro-acids, pyrophosphoric acid ($H_4P_2O_7$) and meta-phosphoric acid (HPO_3), are not reduced to phosphine. Phosphine and hydrogen charged with phosphorus vapor, when ignited, burn with a characteristic green flame (Dusart's reaction):



This green flame coloration may be recognized especially well by holding in the flame a cold porcelain dish or, as in case of the "*chemi-*

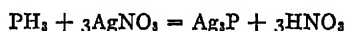
¹ R. Schenck and E. Scharff. A Method for the Detection of Minute Quantities of White Phosphorus. Ber. d. Deutsch. chem. Ges. 39 (1916), 1522.

² Dusart. Note on the Detection of Phosphorus. Compt. rend. 43 (1856), 1126.

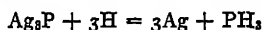
³ Blondlot. Toxicological Detection of Phosphorus by the Flame Coloration. Compt. rend. 52 (1861), 1197.

cal harmonica," over the flame a rather wide glass tube which is then lighted green its entire length. This test is sufficiently delicate, to be distinctly visible even in a dilution of 1 milligram of phosphorus in 15 liters of hydrogen.

A toxicological analysis usually deals with the detection of traces of toxic phosphorus or of its first oxidation product, phosphorous acid. Hydrogen after acting in the nascent state upon the material is not directly examined for phosphorus but is first passed into dilute silver nitrate solution. Phosphine precipitates black silver phosphide (Ag_3P).



Thus traces of yellow phosphorus, or of phosphorous acid, that may be in the cadaveric material, are concentrated in the black silver precipitate from which nascent hydrogen, prepared from phosphorus-free zinc and dilute sulphuric acid, will liberate phosphine.



When ignited, this will then give the Dusart reaction.

The method discovered by Dusart and introduced by Blondlot into forensic chemistry for the detection of minute quantities of phosphorus is based upon these facts.

Silver phosphide is not suitable for the quantitative estimation of phosphorus owing to its slight stability. In the moist condition, especially by the nitric acid resulting from its formation, it is oxidized to phosphoric acid and silver.



Consequently the black precipitate formed by passing phosphine into silver nitrate solution always contains metallic silver together with silver phosphide.

If hydrogen produces a black or gray precipitate in a silver nitrate solution, phosphorus is not necessarily present, since hydrogen sulphide, arsine, stibine and various reducing organic compounds, such as formaldehyde and formic acid, also produce black precipitates. A black precipitate therefore should always be examined for phosphorus by the Dusart reaction. In the detection of yellow phosphorus and phosphorous acid, the Dusart-Blondlot method combines two distinct operations, namely:

1. Preparation of the silver phosphide precipitate.
2. Examination of this precipitate for phosphorus.

I. Preparation of Silver Phosphide

Thin the finely divided material, or the residue remaining in the distillation-flask from the Mitscherlich test, with water in a capacious flask having a doubly-bored stopper through which pass a dropping-funnel and a right-angle exit-tube. Connect the latter with a U-tube containing pieces of pumice-stone saturated with concentrated potassium hydroxide solution to absorb hydrogen sulphide. Attach to this U-tube the absorption-apparatus for phosphine. To insure complete absorption of phosphine by the silver nitrate solution, it is advisable to use a Liebig potash-apparatus employed in the analysis of organic substances, or a Pélégot tube.¹ The absorption-apparatus is partly filled with 3 per cent. neutral silver nitrate solution. Granulated, phosphorus-free zinc is put into the evolution-flask before the material is introduced. Then run in pure dilute sulphuric acid (1:5), until there is a fairly active evolution of hydrogen. If the evolution of hydrogen subsides, more sulphuric acid should be added. Allow the action to continue for 6-8 hours or longer. If the material contains elementary phosphorus, or phosphorous acid, a black precipitate of silver phosphide will appear in the silver nitrate solution. Collect the latter upon a small ash-free paper, wash quickly with a little cold water, and examine for phosphorus as directed below in the Fresenius-Neubauer apparatus, or in that of Hilger-Nattermann, or by heating according to the method of Stieh.

Phosphoric Acid Test.—If the precipitate is silver phosphide, the filtrate will contain phosphoric acid. To test for the latter, first add dilute hydrochloric acid to remove silver from the filtrate. Collect the silver chloride upon a paper previously well-washed with dilute nitric acid and water, and completely expel hydrochloric acid from the filtrate by evaporation upon the water-bath after adding nitric acid. Dissolve the residue in a little water and finally test for phosphoric acid by warming gently with ammonium molybdate solution. If a yellow precipitate appears, collect it upon a small filter, wash, and dissolve in a little ammonia. Add magnesia mixture to the clear solution and note whether a crystalline precipitate of ammonium magnesium phosphate appears (see page 6).

¹ J. Gadamer recommends for the absorption of phosphine two Liebig bulbs connected together, or two Huguershoff wash-bottles with flat glass spirals.

II. Examination of the Silver Precipitate for Phosphorus

In this test the silver precipitate obtained in one of the four following ways may be used·

(1) That obtained direct with silver nitrate in (1) by the action of nascent hydrogen upon the material.

(2) That formed when the distillate by the Mitscherlich method is shaken with silver nitrate.

(3) That obtained in the Fresenius-Neubauer procedure by heating the material in a current of carbon dioxide according to Scherer's method.

(4) That formed in distilling by Scherer's method as modified by Hilger and Nattermann

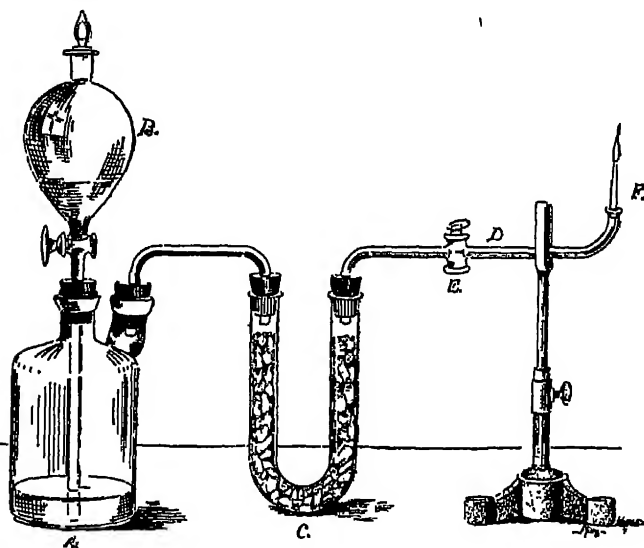


FIG. 4.—Fresenius-Neubauer apparatus

(a) Fresenius-Neubauer¹ Apparatus

Apparatus.—This is shown in Fig 4. Hydrogen is generated in flask (A) from pure phosphorus-free zinc and dilute sulphuric acid. Reservoir (B) serves to hold liquid from (A) when cock (E) is closed. U-tube (C) contains pieces of pumice-stone saturated with concentrated potassium hydroxide solution to absorb any hydrogen sulphide

¹ C R Fresenius Qualitative Chemical Analysis, XVI Edition, page 521.

which interferes with the reaction. A tube of hard glass (D) is provided with a glass stop-cock (E) and a tip¹ (F) of platinum. A platinum tip is absolutely essential, for otherwise the flame instead of being colorless will always be yellow from sodium in the glass. The place where the platinum tip is fused into the glass should be cooled by wrapping cotton around the glass and keeping it moist.

Procedure.—Open (E) and allow hydrogen from (A) to pass through the apparatus for some time to expel air. Then close (E) and the liquid in (A) will rise into (B). Now open (E) just enough to allow hydrogen to burn with a small flame which should be colorless in the dark. If no trace of green is to be seen in the inner cone and a porcelain dish depressed upon the flame does not show an emerald-green coloration, hydrogen is phosphorus-free. It is well to repeat this test. To test for phosphorus in the precipitate obtained with silver nitrate, wash it with the paper into (B) with a little water, making sure that finally all is brought into (A). Then repeat the test in the same manner, that is, close cock (E) and open it when all the liquid in (A) has risen into (B). Now ignite the hydrogen and examine the flame in the dark as above. If the precipitate contains a trace of silver phosphide, the inner cone will be green and a porcelain dish depressed upon the flame will show an emerald-green coloration. Adjust cock (E) so that the hydrogen flame is small and its color may be observed for some time.

(b) Hilger-Nattermann² Apparatus

Reduction takes place in a 100 cc. flask closed by a rubber stopper with three holes, two of which are for right-angle tubes just passing through the stopper. Hydrogen from a Kipp generator is first washed through water and then enters the flask by one tube and leaves by the other. Attach to the latter a U-tube filled with pieces of pumice-stone saturated with concentrated sodium hydroxide solution to absorb hydrogen sulphide. Connect the other end of the U-tube with a hard glass tube tipped with platinum.³ Through

¹ Fresenius and Neubauer use a screw pinch-cock instead of a gas-cock but by means of a short rubber connector they interpose an ordinary cock between gas-flask (A) and U-tube (C).

² H. Nattermann and A. Hilger: *Detection of Phosphorus in Forensic-Chemical Investigations*. *Forschungsbericht über Lebensmittel und ihre Beziehungen zur Hygiene*, 4 (1897), 241-258.

³ Hilger and Nattermann use a platinum-tipped blow-pipe instead of a glass tube tipped with the same metal. Cotton, which is kept moist and acts as a cooler, is wrapped around the blow-pipe below the tip.

the third opening in the stopper a thistle-tube extends to the bottom of the flask. Cut the paper containing the precipitate into small pieces and place in the flask which also contains a few pieces of phosphorus-free zinc and enough water to seal the thistle-tube. Light the hydrogen after it has passed through the apparatus for some time and been found free from air by the usual test. Seen in the dark the flame should be entirely colorless and burn without a green cone or a greenish glow.¹ Hilger and Nattermann advise a spectroscopic examination of the flame to determine the purity of the zinc. Pure zinc gives a hydrogen spectrum showing only an orange-colored line in place of the yellow sodium line. The minutest

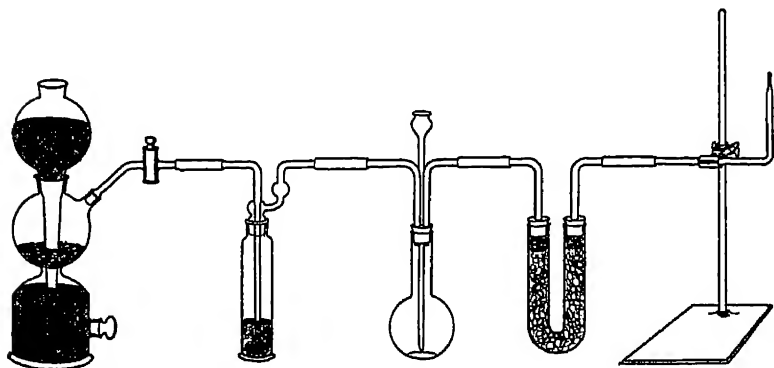


FIG 5 —Hilger-Nattermann apparatus

trace of phosphorus will give three green lines lying to the right of line D. The color of two of these lines is more pronounced than that of the third. Having thus tested the purity of the zinc and sulphuric acid, pour a few cc. of dilute sulphuric acid (1:5) through the thistle-tube into the flask containing zinc and the silver precipitate. If the latter contains phosphorus, the flame will show, though not always at once, a green coloration that should be examined with the spectroscope.

According to Dalmon, the Dusart reaction can be made in the manner of the "*chemical harmonica*" by holding a glass tube over the hydrogen flame and pressing the latter on the inner wall of the glass tube. If phosphorus is present, a beautiful green luminescence will extend throughout the tube and phosphorescent light waves

¹ Zinc entirely free from phosphorus which will stand this test is difficult to obtain.

will appear together with small detonations. If the flame is compressed by lowering the glass tube, a dark blue coloration will appear.

(c) **Decomposition of the Silver Phosphide Precipitate by Heating**
(Stich¹)

Stich decomposes a black silver precipitate not by nascent hydrogen prepared from zinc and sulphuric acid, but by carefully applying gentle heat to the dry precipitate in a current of hydrogen. In this manner phosphine is also liberated from silver phosphide. Stich has hardly ever succeeded in obtaining zinc that is absolutely phosphorus-free. The author has experienced the same difficulty. Various commercial samples of zinc said to be chemically pure, as well as an electrolytic zinc, gave hydrogen that did not exhibit an emerald-green cone in the Dusart-Blondlot tests but thin blue-green streaks. The latter very decidedly interfered with the recognition of phosphorus in the Dusart-Blondlot test, if they did not make it quite impossible. For this reason Stich first passes hydrogen through glass-wool moistened with 3 per cent silver nitrate solution, thereby getting a hydrogen flame that is absolutely colorless.

The black silver precipitate is collected upon a plug of pure asbestos in an Allihn filter-tube, washed and dried with gentle heat or in a vacuum-desiccator. The hydrogen obtained from zinc and dilute sulphuric acid in a Kipp generator is first passed through 3 per cent silver nitrate solution to render it phosphorus-free and then dried with concentrated sulphuric acid. On the other side the Allihn filter-tube, connected with a U-tube containing pieces of pumice-stone saturated with potassium hydroxide solution, terminates in a platinum-tipped glass tube (Fig. 6). Hydrogen is first passed through the apparatus to displace air, then lighted and the suspected silver phosphide precipitate in the Allihn tube heated with a very small flame. If the black precipitate contains silver phosphide, the latter is decomposed with formation of phosphine producing a green cone in the flame. Iron obtained by igniting ferrous oxalate in a current of hydrogen may also be substituted for absolutely pure zinc.

Notes.—According to Nattermann and Hulger, 1/10 or at most 1/5 of the phosphorous acid is reduced to phosphine in the Dusart-Blondlot method. The

¹ C. Stich: Formation of Gaseous Compounds of Phosphorus in Putrefaction. *Mitteilungen a. d. analyt. Laborat. d. Krankenhausapotheke zu Leipzig*

delicacy of the Dusart-Blondlot method is very great. By this flame test even $1/10,000$ of a milligram of phosphorus is said to be recognizable (Nattermann and Hilger). Oxidation of phosphorus in a mixture of organic substances of varying composition such as food residues, or in any organic material in process of decomposition, sometimes proceeds rather slowly. Putrefactive processes in particular more or less prevent oxidation of phosphorus. Even after six months 3 milligrams of phosphorus were detected in organic material that had entered into putrefaction (Nattermann and Hilger).

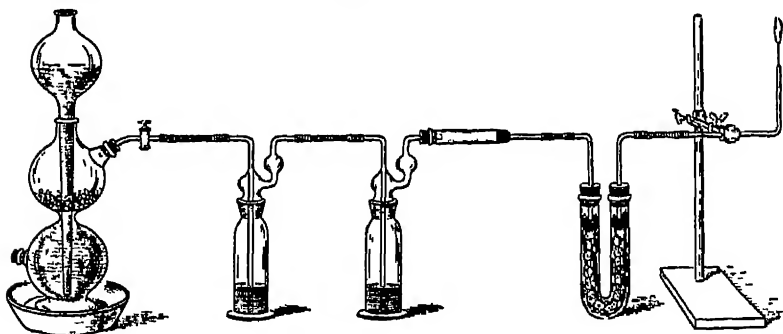


FIG 6—Apparatus of Stich.

Absorption of Phosphine by Cuprous Chloride

(Hilger and Nattermann)

To absorb phosphine, Hilger and Nattermann use a hydrochloric acid solution of cuprous chloride, first recommended by Ribau¹ for estimating PH_3 in gas mixtures. A molecular compound of the two substances is formed but at 70° is again resolved into its components.

Dissolve 4 grams of pure cuprous chloride in 20 cc. of hydrochloric acid (sp. gr. 1.19 = 37.5 per cent.) and add 30 cc. of 12 per cent. potassium hydroxide solution. After about 4 hours decant the clear solution from the crystals and put 20 cc. of this solution in each of two wash-bottles that are connected. Between the flask containing the material to be examined together with zinc and dilute sulphuric acid and one of the two wash-bottles containing the cuprous chloride solution is introduced an empty flask. The tubes by which the gas enters and leaves the latter end just below the stopper, to prevent cuprous chloride solution from getting back into the evolution-flask. After the action has proceeded for several days (14 days according to Hilger and Nattermann), pour the hydrochloric acid-cuprous chloride solution from the two wash-bottles into a boiling-flask provided with a doubly bored stopper through which two right-angle tubes pass. These tubes end just below the stopper. One serves as the entrance-tube for hydrogen prepared in a Kipp generator from pure zinc and dilute sulphuric acid and washed through water. The other as the exit-tube is connected with a U-tube containing pieces of pumice-stone saturated with potassium hydroxide.

¹ Ribau: Comptes rend. 88 (1879), 11

solution. The latter is attached to a glass tube tipped with platinum. As soon as hydrogen has completely displaced the air in the apparatus, it is lighted and tested for phosphorus. If found free from phosphorus, the cuprous chloride solution is warmed upon the water-bath. At about 70° phosphine begins to come off, mixed with hydrogen, and imparts the characteristic green coloration to the flame.

Detection and Estimation of Phosphorus by Combined Methods of Scherer and Dusart-Blondlot in Fresenius-Neubauer Procedure¹

This method is based upon the volatility of yellow phosphorus in a stream of carbon dioxide from material acidified with sulphuric acid, the precipitation by silver nitrate of phosphorus carried over in the distillate, and its detection in the black precipitate formed by means of the Dusart reaction. This method is very useful for the quantitative estimation of phosphorus in any material whatever.

Procedure.—Distillation is conveniently carried out in the apparatus shown below (Fig 7). Comminute the material as finely as

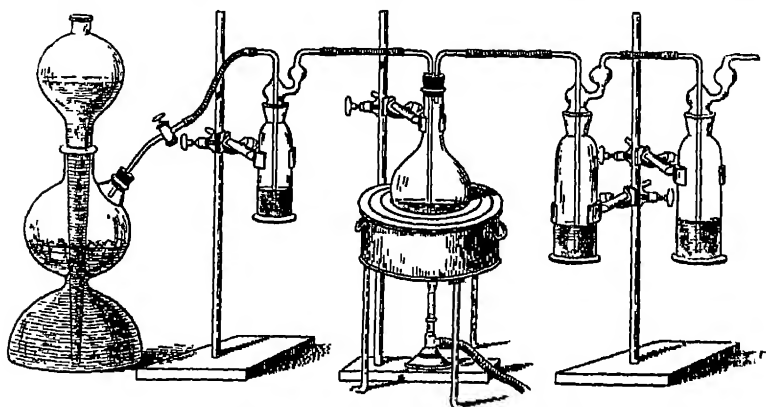


FIG 7—Fresenius-Neubauer apparatus for quantitative estimation of phosphorus

possible, introduce into a capacious but not too large flask, if necessary with addition of a little water, and acidify with dilute sulphuric acid. The flask is provided with a doubly bored stopper through one opening of which a right-angle tube, extending nearly to the bottom, serves for the introduction of gas. The right-angle tube passing through the other opening ends just below the stopper and is in connection with two absorption-bottles one-third full of 3 per

¹ *Zeitschrift f. analyt. Chem.* 1 (1862), 129

cent silver nitrate solution. A Pélégot-tube may also be used to hold the silver nitrate solution. Carbon dioxide from a Kipp generator is washed through water and passed through the apparatus to remove air as completely as possible which may require 20-30 minutes. Then, while keeping up the stream of carbon dioxide, warm the flask to 60° , or at most to 70° , so that too much water vapor is not carried over into the receivers. In a qualitative test for phosphorus it is sufficient to pass carbon dioxide through for 2-3 hours. In a quantitative experiment carbon dioxide should be passed through until the vapors that issue when the apparatus is opened at the place where the gas-mixture enters the absorption-bottles no longer exhibit phosphorescence in a darkened room. As a rule, if the current of carbon dioxide is rapid, 50 milligrams of phosphorus will pass over in 8-10 hours. For a qualitative test collect the black precipitate upon a small filter washed with dilute nitric acid and water, rinse with a little water and examine for phosphorus in the Dusart-Blondlot apparatus or, in case the precipitate is in an asbestos filter-tube, by the method of Stuch.

The filtrate from the precipitate of silver phosphide always contains phosphoric acid. To detect it, remove excess of silver with hydrochloric acid, evaporate the filtrate upon the water-bath, and test the solution of the residue in a little dilute nitric acid by warming it at about 40° with ammonium molybdate solution. For the quantitative estimation of phosphorus, evaporate the contents of the absorption-bottles upon the water-bath with an excess of aqua regia, extract the residue with a little hot water containing a few drops of dilute nitric acid, filter, saturate the filtrate evaporated to about 10 cc with ammonia, precipitate phosphoric acid with clear magnesia mixture, and finally weigh as magnesium pyrophosphate, $Mg_2P_2O_7$.

Notes.—The Fresenius-Neubauer procedure is especially recommended, if the material contains substances that interfere with phosphorescence in the Mitscherlich apparatus. In such a case the distillate obtained by the Mitscherlich method may be shaken with silver nitrate solution, or the distillate may be caught in silver nitrate solution, and a black precipitate tested for phosphorus by the Dusart-Blondlot or Stuch method. In quantitative experiments the author has repeatedly determined phosphorus in the black precipitate and phosphoric acid in the filtrate that came from the latter. As a result it has been found that 30-40 per cent. of the total phosphorus was present as phosphoric acid in the filtrate from the phosphorus precipitate.

Detection and Estimation of Phosphorus by Mitscherlich-Scherer Method Modified by Nattermann and Hilger¹

By a convenient modification Nattermann and Hilger have combined the Mitscherlich method of detecting phosphorus with that of Scherer, thus making it possible to produce the very characteristic phosphorescence as well as to obtain as large a quantity of phosphorus as possible in the distillate and to distil in a current of carbon dioxide. This procedure is always to be recommended, if a qualitative test for phosphorus is to be made in conjunction with a quantitative estimation

Procedure.—Distillation is conducted in the apparatus of Nattermann and Hilger shown below (Fig. 8) In order to produce phosphorescence in the horizontal part of the Mitscherlich air-cooler not far from the place where it becomes perpendicular and enters the evolution-flask, Nattermann and Hilger introduce a 30 cm. glass tube provided with a rubber connector and screw-clamp or glass stop-cock The receivers are an Erlenmeyer flask tightly connected with the condenser and a glass cylinder. Both contain 3 per cent silver nitrate solution Gadamer uses a Pélignot-tube instead of the cylinder. These receivers are connected with a suction-pump As soon as the apparatus is ready, pass through the flask, containing the comminuted material acidified with tartaric acid or dilute sulphuric acid, for at least half an hour a gentle stream of carbon dioxide washed through water so that the entire apparatus is free from air Then gradually heat the flask containing the material in an air-bath or paraffine-bath all the while maintaining the stream of carbon dioxide. As soon as the doubly bent right-angle tube connecting the flask and condenser begins to get warm, start the suction-pump and at once admit air by means of the screw-clamp or glass stop-cock If only traces of phosphorus vapor are present in the distillation-tube and condenser, brilliant phosphorescence will appear as soon as they meet the air that is drawn in. Phosphorescence is said to appear even in presence of those volatile substances that prevent it entirely in the Mitscherlich apparatus (A. Fischer) Having observed phosphorescence, close the clamp or stop-cock and continue distillation until a further test with air

¹ H. Nattermann and A. Hilger. Detection of Phosphorus in Forensic-Chemical Investigations. Forschungsberichte über Lebensmittel und ihre Bez. z. Hygiene 4 (1897), 241

is negative To determine phosphorus quantitatively, evaporate the contents of the receivers, that is, solutions and precipitates, in a porcelain dish upon the water-bath with a little aqua regia. Dissolve the residue in water containing hydrochloric acid, filter from silver chloride, precipitate phosphoric acid in the filtrate with clear magnesia mixture, and finally weigh as magnesium pyrophosphate, $Mg_2P_2O_7$.

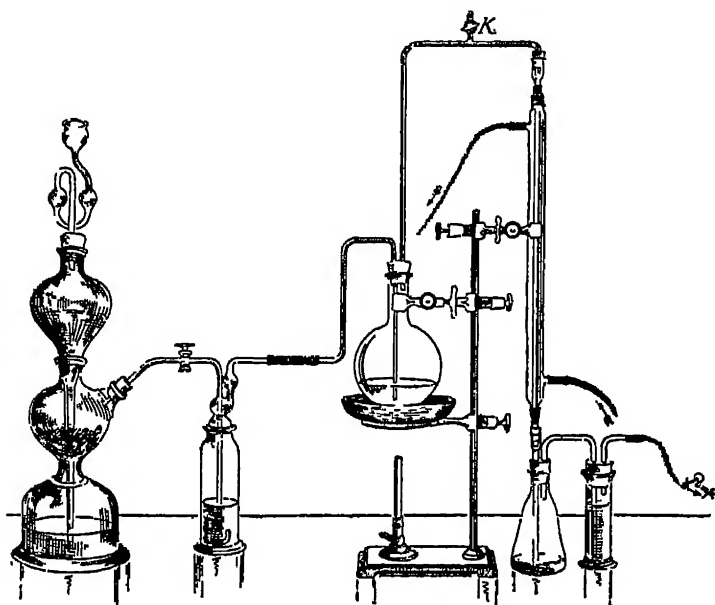


FIG 8—Hilger-Nattermann apparatus for detecting and quantitatively estimating phosphorus

Notes.—Obviously the black silver precipitate may be used for the Dusart reaction, in case this test is necessary. By their procedure Nattermann and Hilger obtained in the distillate 92.4–95.1 per cent of the phosphorus present. In accuracy it is hardly inferior to Scherer's procedure and gives moreover the very characteristic phosphorescence.

Are Volatile Phosphorus Compounds Formed in Putrefaction and by Action of Nascent Hydrogen upon Normal Phosphorus Compounds in the Animal Organism?

Opinions of different investigators, who have turned their attention to this question, are not in accord

According to Stich,¹ gases containing phosphorus are formed in certain putrefactive processes of animal and plant substances. What the nature of these gases is cannot be determined because they are formed in such small quantity. The putrefactive bacteria used were in part bacteria *Coli communis* and in part mixed cultures, that is, from putrefying pancreatic juice. The culture-media consisted of alkaline solutions (1 percent each) of glucose and protein, that is, peptone, casein, sodium caseinate, nuclein, lecithin from the yolk of egg and protagon. Stich also subjected a number of animal and plant substances, especially internal organs in the fresh condition, to putrefaction at 37°. The gases formed in the putrefaction of protein substances were free from phosphorus and the residue from putrefaction gave none of the reactions that indicate the lower stages of oxidation of phosphorus. But it is true that phosphoric acid was detected in the residue from the putrefaction of casein, nuclein, lecithin and protagon. These substances also gave phosphoric acid as a result of the action of nascent hydrogen. According to Stich, gases containing phosphorus are obtained as a result of the action of nascent hydrogen from putrefying fish, brain, potatoes, and also when these are fresh. Upon the basis of his experimental results Stich maintains that only the detection of elementary phosphorus should be accepted as indisputable proof of phosphorus poisoning. A. Fischer² was unable to confirm the results of Stich's experiments wherein gases containing phosphorus were obtained from putrefying potatoes as well as from fresh potatoes as a result of the action of nascent hydrogen. Nor was he able to establish the fact that such gases were given off from putrefying brain. Previously Z. Halász³ had obtained results identical with those of Fischer. He examined two kinds of animal material by the Dusart-Blondlot method. First, he tested normal brains (man, calf, hog), second, the brain and other organs of rabbits that had been given poisonous doses of phosphorus by the mouth and subcutaneously. He examined these organs when fresh and also from week to week after more or less pronounced putrefaction had set in, but could not detect phosphorus in the brain in a single instance. These experiments disprove the earlier idea that phosphorus normally present in the brain is so changed during putrefaction that it can be detected by the Dusart-Blondlot method. He also failed to detect phosphorus or phosphine in the brain of rabbits poisoned by this element, though he found it in other organs, such as stomach and intestines, and in those rich in blood, such as liver, lungs and kidneys. He could always detect small or large quantities of phosphorus in any organ which this element had directly reached, or by which it had been indirectly absorbed. Halász concludes from his experiments that if any volatile phosphorus compound is formed in the brain during putrefaction, it is not volatile with steam but with alcohol and does not give the Dusart-Blondlot reaction. This amounts to saying that such a compound cannot be detected by the forensic-chemical method. On the basis of these experiments Halász holds

¹ C. Stich. Formation of Gaseous Compounds of Phosphorus in Putrefaction. *Mitt. a. d. analyt. Laborat. zu Leipzig* 22 (1900).

² A. Fischer: Contributions to the Detection of Phosphorus. *Pföger's Archiv. der Physiologie* 97 (1903), 578.

³ Z. Halász. Is the Blondlot-Dusart Method reliable in Forensic-Chemical Cases? *Zeitschr. f. anorg. Chemie* 26 (1900), 438.

that the Dusart-Blondlot method of detecting phosphorus is just as reliable for forensic purposes as that of Mitscherlich.

Duration of Yellow Phosphorus in the Cadaver

Yellow phosphorus can be detected after a considerable time in any organic matter whatever, even when it has reached a high state of putrefaction. Processes of putrefaction and decay prevent or at least retard the oxidation of phosphorus. In the cadaver phosphorus is also only slowly oxidized. This fact may be explained when it is recalled that atmospheric oxygen is necessary for the oxidation of phosphorus and that during putrefaction reducing gases that protect phosphorus from oxidation are formed. For this reason after poisoning phosphorus frequently can be detected as such in the cadaver after a rather long time. Dragendorff detected phosphorus in an exhumed body several weeks after death. Elvers found free phosphorus in a human body eight weeks after death. Bischoff found phosphorus after six months and Poleck¹ even earlier had proved the presence of traces of free phosphorus in a cadaver three months after poisoning had occurred. Recently Alpers² found even 13.9 mg. of elementary phosphorus in the gastro-intestinal contents of a woman who had died as a result of acute phosphorus poisoning and whose body was not exhumed until four weeks after death. In this investigation Alpers observed that phosphorus distils over very slowly even in a rapid current of steam. Distillation of phosphorus did not come to an end until after about 8 hours.

Absorption and Toxic Action of Phosphorus

Absorption of phosphorus takes place from every mucous surface and wound. The form in which phosphorus is absorbed is a question that cannot be answered with certainty. In view of the powerful, specific toxic action of phosphorus, however, it must be assumed that it gets into the circulation dissolved in water, bile or fat, or in the form of vapor. This hypothesis as to the absorption of phosphorus in the elementary condition finds support in the fact that after poisoning by phosphorus it can be detected as such in the liver, in the blood and in expired air. In this connection mention should be made of the retention of phosphorus as such unchanged in the blood for hours and even for days. Phosphorus dissolved in oils is held at ordinary temperature by arterial as well as by venous blood, but not by serum. Phosphorus can be detected both chemically and spectroscopically in expired air from animals poisoned by phosphorus. In case of poisoning by large intra-arterial doses of phosphorus, the expired air is highly ionized. Consequently in such cases, elementary phosphorus gets from the blood into the alveolar wall where it is oxidized (Schmidt³).

Evidences of phosphorus poisoning in many cases do not appear for some time, occasionally not for several hours after the phosphorus has been taken, in very

¹ Th. Poleck. Detection of Phosphorus Poisoning in a Cadaver three months after Death. *Archiv. d. Pharmazie* 225 (1887), 189.

² K. Alpers: Detection of Elementary Phosphorus in a Cadaver four weeks after Death. *Pharm. Ztg* 58 (1913), 127.

³ H. Schmidt. A Contribution to our Knowledge of Phosphorated Oils, and their Retention in the Organism, by means of the Electroscopic Detection of Phosphorus. *Biochemische Zeitschr* 34 (1911), 280.

rare instances not for one or two days. In the gastric region there is a burning sensation with pains and, preceded by belching and choking, there ensues vomiting of masses that are luminous in the dark and smell of phosphorus. The exhaled air has a more or less strong smell of phosphorus. Usually there is severe burning in the throat and diarrhoea in about 30 per cent of the cases. The liver begins to swell and as it increases in size, the face usually on the second or third day after the poison has been taken shows the characteristic icteric discoloration which may confine itself to that area or extend over the entire body. Additional occurrences in acute phosphorus poisoning are: nose-bleed, bleeding from the intestines and in women from the genitals, muscular paralysis, ringing in the ears, even deafness, swimming of objects before the eyes, and dullness of vision. The pulse is irregular, the patients become stupefied, delirious, quiet and end in a state of coma (Lewin).

The average lethal dose of phosphorus is from 0.1-0.2 gram, although a quantity as small as 0.05 gram has given rise to fatal poisoning. On the other hand, much larger doses of 0.3-0.5 gram of phosphorus, especially if taken in one piece, have produced only milder symptoms of intoxication. As a rule death ensues within six days and in most cases on the second or third day, in isolated cases 79 hours after the poison was taken.

In a case of suspected phosphorus poisoning, gastric and intestinal contents, blood, and organs rich in blood, such as the liver, should be examined. Vomitus is also an important material for examination.

Metabolism in Phosphorus Poisoning

In very small daily doses of 0.5-1 milligram, phosphorus exerts in man an increasing influence upon metabolism, that is, upon growth and formation of new tissue. Physicians have come to this conclusion from observing clinically the favorable influence upon the condition of nutrition as a whole and the unusual increase in weight of children treated with phosphorus for rachitis. (Kassowitz, Hagenbach 1884.) The action of phosphorus in man in the beginning causes an increase in the number of red blood-corpuscles and also exerts a favorable influence upon bone-formation. In larger quantities phosphorus has a very poisonous action upon the processes of metabolism. Under its influence a marked increase in the breaking down of tissue ensues together with accompanying disturbances of the processes of oxidation, synthesis and decomposition. In phosphorus poisoning the normal course of chemical metabolism undergoes complete derangement. The consumption of oxygen as well as the formation of carbon dioxide is diminished; less fat is decomposed but so much the more carbohydrate and protein; the latter, however, is only incompletely broken down so that products indicating partial decomposition, such as peptones, amino-acids and lactic acid are found in blood and urine.

In consequence of incomplete combustion of fat, it is deposited in the organs in large quantity. This is distinctly noticeable in the liver, heart, kidneys, partly also in the diaphragm and in the other muscles which collectively give more or less evidence of fatty degeneration. In the liver and heart it is probably also a question as to the transference of fat from other tissues, it may arise in part from the subcutaneous deposit.

The decomposition of protein, as stated above, shows a marked increase in phosphorus poisoning. The protein of organs is broken down more extensively but not as completely as in normal protein metabolism. Porges and Pribram¹ investigated protein exchange in the liver of animals poisoned with phosphorus and found that the phosphorus liver just removed from the animal was relatively poorer in protein than the normal liver. Autolytic cleavage of protein in the phosphorus liver as contrasted with autolysis in normal livers is materially increased. In phosphorus poisoning as in respiration in air very poor in oxygen decomposition of body tissues shows an increase with partial formation of such nitrogenous and non-nitrogenous products of metabolism as normally either are not formed at all in the organism or appear merely as intermediate products of oxidative animal metabolism. Decomposition of the protein molecule goes in part only as far as the amino-acids. Consequently in phosphorus poisoning the urine almost always contains leucine, $(\text{CH}_3)_2\text{CH}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$, tyrosine, $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$ (1.4), and also sarco-lactic acid, $\text{CH}_3-\text{CH}(\text{OH})-\text{COOH}$. Acids that normally occur in the urine only in traces may be detected in much larger quantity in acute phosphorus poisoning. Such acids are p-oxyphenyl-acetic acid, $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2-\text{COOH}$ (1.4) and p-oxyphenyl-propionic acid, or hydropara-cumic acid, $\text{HO}-\text{C}_6\text{H}_4-\text{CH}_2-\text{CH}_2-\text{COOH}$ (1.4). Cystine, $\text{HOOC}-\text{CH}(\text{NH}_2)-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$, has also been repeatedly found in phosphorus urine.

In phosphorus poisoning there is a marked decrease in the urea-content of the urine but a decided increase in total nitrogen. A considerable part of the nitrogen, that is, 25 per cent. or more of the total nitrogen, appears to leave the body as ammonia, whereas the urine of adults usually contains from 2-5 per cent. of the total nitrogen as ammonia. This increase of ammonia may have some connection with the increase in formation of acid during phosphorus poisoning, since the ammonia serves to neutralize the acid formed.

Peptone-like substances, the presence of which is attributed to profound disturbance of metabolism, frequently appear in the urine in phosphorus poisoning. Various observers believe that there is no longer any doubt as to the appearance of genuine peptonuria. A glycosuria may also appear, or be easily induced by a diet rich in sugar. In accord with this observation is the fact that the liver of an animal poisoned by phosphorus is without the power to change glucose of the blood into glycogen and store up the latter. Since persons poisoned by phosphorus have icterus (jaundice), bile-pigment, or an abundance of urobilin, can almost always be detected in the urine.

The disturbances of metabolism evoked by phosphorus coincide in many respects with the anomalies that arise from deficiency of oxygen. Consequently it is possible that the cells of the body poisoned by phosphorus are less capable of utilizing normally the oxygen that is taken up. This is shown by the fact that the amounts of oxygen and carbon dioxide, which the organism respectively takes up and gives off, show a marked diminution during phosphorus poisoning. Only 48 per cent. of carbon dioxide, as compared with 100 per cent. under normal

¹ O. Porges and E. Pribram. Contribution to the Knowledge of the Chemical Processes in Phosphorus Poisoning. *Archiv f. exp. Pathol. u. Pharmac.* 59 (1908), 20.

conditions, may be eliminated. Although the phosphorus that gets into circulation does not reduce the oxyhaemoglobin of the blood, it increases its power of transferring oxygen to oxidizable substances. The free phosphorus in the animal organism is only very gradually oxidized to phosphorous acid and finally to phosphoric acid.

Urine secreted during phosphorus poisoning has a strong acid reaction, usually containing lactic acid, almost always protein or peptone-like substances, and the above mentioned amino-acids, as well as fat-cylinders, cell-detritus, free fat globules and blood-corpuscles.

Oxidation of Phosphorus, Luminescence and Electrical Conductivity of Phosphorus Air

Schenck, Mihr and Banthuen,¹ as well as Scharff,² have thoroughly investigated the relations existing between oxidation of phosphorus, luminescence and electrical conductivity of phosphorus air and arrived at the following results. White phosphorus is first oxidized to phosphorus trioxide, P_4O_6 , without the appearance of luminescence, the latter not appearing until further oxidation takes place. In moist oxygen the luminescence of phosphorus trioxide is strongly marked and very beautiful, whereas it fails to appear in absolutely dry oxygen. In this case is very distinctly shown the phenomenon of "intermittent luminescence" and its dependence upon the proportion of oxygen in the mixture. Since phosphorescence depends upon the presence of oxygen and water vapor, that is, upon moist air, and since it has further been uniformly shown that conductivity in presence of white phosphorus takes place only while processes of oxidation are going on, obviously all factors checking oxidation will prevent luminescence as well as the electrical conductivity of phosphorus air. Complete parallelism exists between the appearance of the oxidation luminescence and the ionization of phosphorus air, for these phenomena cease as soon as the vapor of oil of turpentine, alcohol, ethylene, mesitylene, ammonia, carbon disulphide, or illuminating gas is added to the air before it passes over the phosphorus.

Various oxidation phenomena of entirely different substances are quite similar to those of phosphorus. Sulphur, for example, at 200° undergoes slow oxidation and exhibits phosphorescence. At the same time there appears an oxidation product smelling like camphor and very irritating to the eyes (Haumann). Brom-acetylene, CH_2Br , which Nef regards as brom-acetylene, $CHBr:C$, is said to behave very much like phosphorus. Water and alcohol dissolve it in small quantities, its solutions phosphoresce in the dark just as phosphorus does and exhibit a strong ozone reaction and a distinct ozone smell. In its physiological action brom-acetylene is said to be very similar to phosphorus. According to Schenck ozone, phosphorus pentoxide and its hydrates cannot be considered as exciters of electrical conductivity, but probably vapors of phosphorus trioxide have an ionizing action upon air. The action of the vapor of phosphorus trioxide

¹ R. Schenck, F. Mihr, H. Banthuen: The Component of Phosphorus Air causing Electrical Conductivity. *Ber d Deutsch chem. Ges.* 39 (1906), 1508.

² E. Scharff. Luminescence of Phosphorus and of some Phosphorus Compounds. *Zeitschr. f. physikal. Chemie* 62 (1908), 179.

upon the charged electroscope is especially strong so that there can be no doubt that this trioxide is the exciter of the electrical conductivity of phosphorus air

Detection of Phosphorous Acid in the Cadaver

The reduction of phosphorous acid to phosphine by zinc and dilute sulphuric acid takes place very slowly Hilger and Nattermann (loc cit) state that even a few milligrams require the action of nascent hydrogen for 10-14 days. Moreover careful manipulation is necessary because silver phosphide is quite unstable. Water decomposes this substance into metallic silver and phosphorous acid and the nitric acid present oxidizes the latter to phosphoric acid. Therefore in a toxicological examination, if special attention must be given to phosphorous acid, Hilger and Nattermann recommend examining the silver precipitate (presumably Ag_3P) after 2 days, or at most 3, for phosphorus by the Dusart-Blondlot method and the filtrate for phosphoric acid (see p 17)

Hypophosphites.—Various hypophosphites, such as calcium hypophosphite, $\text{Ca}(\text{H}_2\text{PO}_2)_2$, are often used medicinally, for example, in the treatment of children. With nascent hydrogen these compounds yield phosphine and consequently in the absence of phosphorus they may lead to the conclusion that it is present. Hypophosphites according to Th. Panzer¹ are so quickly eliminated that 24 hours after their administration they can no longer be detected in the organism. Consequently a positive result with the Dusart-Blondlot reaction only indicates phosphorus poisoning when it can be shown that the individual did not take hypophosphites internally on the day before his decease. Calcium hypophosphite can be detected in the urine 15 minutes after it has been taken. Such urine imparts a distinct green coloration to the flame when examined by the Dusart-Blondlot method. Apparently this salt passes through the organism without being in any way retained (Panzer)

Examination for Other Volatile Poisons except Phosphorus

When phosphorescence during distillation has been distinctly observed in the Mitscherlich apparatus, either this operation may be continued, or it may be stopped and distillation resumed with the Liebig condenser in its customary position (Fig. 9). This simpler method of distilling should always be used, when Scherer's preliminary test for phosphorus is negative, or when there is no occasion to test for phosphorus.

Since the several poisons that may appear here are not equally volatile with steam, it is best to collect the distillate in two or three fractions. The first fraction will contain most of the easily volatile substances, such as hydrocyanic acid, chloroform, alcohol, methyl alcohol, iodoform and nitrobenzene. The other fractions may

¹ Th. Panzer Behavior of Calcium Hypophosphite in the Animal Body. *Zeitschr. f. Unters. d. Nahrung- und Genussm.* 5 (1902), 11.

contain considerable quantities of such substances as carbolic acid, aniline, chloral hydrate, formaldehyde and carbon disulphide that volatilize less easily with steam and so distil over more slowly. This must not be understood to mean that the first part of the distillate will be free from substances that volatilize with difficulty, and the latter part free from those that volatilize easily. In the main such will be the separation, but either part of the distillate may contain traces of substances that will appear in larger quantity in the other part.

The correct procedure is to distil until 5-10 cc. of liquid have been collected. Divide this distillate into several portions and test

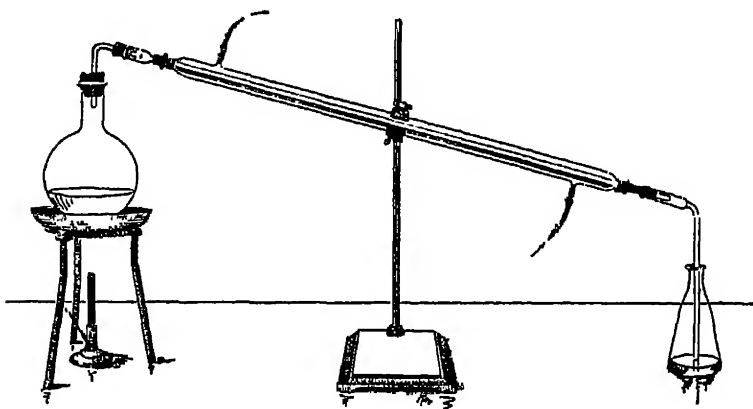


FIG 9 —Simple distillation apparatus with Liebig condenser

for hydrocyanic acid, chloroform and alcohol, and, if necessary, also for iodoform and nitrobenzene. Then distil off 10-20 cc. more and use this distillate to test for carbolic acid, cresols, aniline, chloral hydrate, formaldehyde, carbon disulphide and other volatile substances.

Several of these volatile substances have a characteristic odor, such as carbolic acid, nitrobenzene, alcohol, chloroform, iodoform and hydrocyanic acid, which makes it possible to recognize them with great certainty in the original material and especially in the distillate, provided more than traces are present. First, test the distillate for each individual substance by its most characteristic reaction. Test for hydrocyanic acid by the Prussian blue or sulphocyanate reaction; for volatile phenols and aniline with Millon's reagent; for

alcohol, acetone and acetic aldehyde with iodine and potassium hydroxide solution, for chloroform, iodoform and also chloralhydrate with aniline and potassium hydroxide solution, that is, by the phenyl-isocyanide reaction, and finally for carbon disulphide with lead acetate and potassium hydroxide solution. If there is reason to believe that a volatile poison is present, confirm the result by making other characteristic tests. It is seldom necessary to examine the distillate for all the members of the group.

HYDROCYANIC ACID

Hydrocyanic acid, HCN, and various cyanides, such as potassium cyanide, KCN, and sodium cyanide, NaCN, belong to those poisons frequently used in poisonings and suicides because their swift and sure action is generally known. Inasmuch as cyanides of the alkalis are frequently used in electro-metallurgical processes, metallurgy and photography, they are quite easily accessible. Improper medicinal use of bitter almond water and cherry laurel water has also led to poisonings. Both of these preparations contain 0.1 per cent. of hydrocyanic acid, present in part as the cyanohydrin of benzaldehyde. The ferro- and ferri-cyanides, such as yellow and red prussiate of potash, as well as Prussian blue, are practically non-toxic, for with the dilute acid of the gastric juice at body-temperature they give at most only traces of hydrocyanic acid. Usually genuine "Kirschwasser"¹ and "Zwetschgenwasser"² also contain hydrocyanic acid. By the colorimetric method the author has found 30 milligrams of hydrocyanic acid in 100 cc. of genuine Black Forest "Kirschwasser." Tobacco smoke frequently contains traces of hydrocyanic acid. According to Habermann,³ it is not present in pipe smoke, whereas the smoke of Austrian cigarettes contains 0.0023-0.0037 per cent. of hydrocyanic acid and that of cigars 0.0012 per cent. Pontag⁴ by smoking 100 grams of Russian tobacco obtained 8 milligrams of hydrocyanic acid and Thoms⁵ by smoking

¹ Cherry brandy.

² Plum brandy.

³ Habermann. Contributions to the Knowledge of Cigar Smoke. *Zeitschr. f. physiol. Chemie* 33 (1901), 55 and 40 (1904), 148.

⁴ J. J. Pontag. Investigation of Russian Tobacco Smoke and of Cigarette Smoke. *Zeitschr. f. Untersuchung d. Nahrungs- und Genussmittel* 6 (1903), 673.

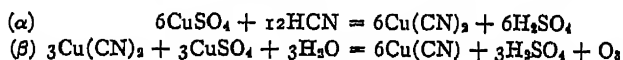
⁵ H. Thoms: The Products in Tobacco Smoke. *Ber. d. Deutsch. pharm. Ges.* 10 (1900), 19.

15 of Wendt's patent cigars, having a total weight of 73 grams, obtained 3.4 milligrams of Prussian blue

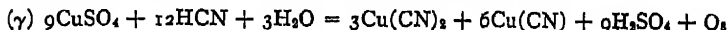
In a chemical examination for hydrocyanic acid and simple cyanides, such as sodium or potassium cyanide, the contents of the stomach and intestines of the cadaver are most important. In addition organs rich in blood, such as liver, brain and heart, the blood itself and sometimes the urine are also taken. Examine such material at once for hydrocyanic acid which may be recognized by its characteristic odor, provided putrefaction has not gone too far. First make the following preliminary test.

Preliminary Test.—A special test (Schönbein-Pagenstecher) for hydrocyanic acid should precede distillation. Acidify a portion of the original material in a small flask with tartaric acid solution. Then suspend in the flask (see Fig 1, page 2) a strip of "guaiac-copper" paper¹ without letting it touch the liquid. Gently warm the contents of the flask upon the water-bath. Neither hydrocyanic acid nor potassium cyanide is present, unless the paper is turned blue or bluish green. But the only conclusion to be drawn from a positive test is that hydrocyanic acid, or an easily decomposable cyanide, may be present. Further conclusions should not be drawn from a positive result, since other substances such as ammonia, volatile ammonium compounds, hydrochloric acid, and especially oxidizing agents such as ozone, hydrogen dioxide, nitric acid, and chlorine will turn the paper blue. Consequently, though very delicate, this test cannot be accepted as conclusive proof of the presence of hydrocyanic acid.

The ozonizing action of hydrocyanic acid in conjunction with copper sulphate may be explained by the following equations:



These two equations taken together are as follows:



To apply the very delicate guaiac test in detecting hydrocyanic acid in distillates, add first to the distillate a drop of freshly prepared, dilute alcoholic guaiac tincture and then a drop of copper sulphate solution. If the distillate

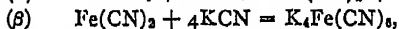
¹ Prepare "guaiac-copper" paper by saturating strips of filter paper with freshly prepared, 10 per cent alcoholic tincture of resin of guaiac. Dry these strips in air and moisten before using with very dilute aqueous copper sulphate solution (1:1000).

contains hydrocyanic acid, the mixture will turn deep blue, the coloration reaching a maximum at once. After some time the intensity of color of the blue solution considerably diminishes.

The actual chemical examination for hydrocyanic acid is made by adding tartaric acid solution to the finely divided material and distilling as described (see p 26). This acid volatilizes easily with steam and most of it will appear in the first part of the distillate. Therefore use the first 10-15 cc of distillate for the tests. Note cautiously the odor of the distillate, which is characteristic, and then proceed as follows.

1. Prussian Blue Test.—Add to the solution (distillate) a little potassium hydroxide solution, then 1-2 drops of freshly prepared ferrous sulphate solution and 1 drop of ferric chloride solution. Shake well and warm gently. Finally acidify with dilute hydrochloric acid. If much hydrocyanic acid is present, a precipitate of Prussian blue will appear at once. But if the quantity is small, the colloidal solution will have merely a blue, blue-green or green-blue color. After a long time (10-12 hours) a flocculent precipitate of Prussian blue will settle to the bottom of the test-tube. The limit of delicacy of this test is 1:50,000¹.

Explanation.—Hydrocyanic acid and potassium hydroxide form potassium cyanide which with ferrous sulphate produces ferrous cyanide (α). The latter combines with more potassium cyanide, forming potassium ferrocyanide (β) which with ferric chloride precipitates Prussian blue (γ), the ferric salt of hydroferrocyanic acid, $H_4Fe(CN)_6$.



Prussian blue will not appear in presence of alkalis, since they decompose it as follows:



Consequently test the final mixture with blue litmus paper, to make sure that it is acid.

Notes.—According to Vorländer,² too much hydrochloric acid retards the formation of Prussian blue. In concentrated hydrochloric acid Prussian blue is

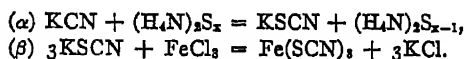
¹ A. Link and R. Möckel: Detection of Hydrocyanic Acid. Zeitschr. f. analyt. Chem 17 (1878), 455

² D. Vorländer The Prussian Blue Reaction Ber. d. Deutsch. chem. Ges 46 (1913), 181

even soluble with yellow color. If, however, the concentration of the acid is kept below 0.5 normal, its influence upon the formation of Prussian blue may be disregarded. The double decomposition of ferric salts with potassium ferrocyanide in aqueous solution is a time reaction which is retarded or prevented by acids and salts. Therefore the Prussian blue formation does not depend upon a simple instantaneous ionic reaction (Vorländer)

2. Sulphocyanate Test.—Add to a portion of the distillate a few drops of potassium hydroxide solution and then a little yellow ammonium sulphide solution. Evaporate this mixture to dryness in a porcelain dish upon the water-bath. Dissolve the residue in a little water and acidify with dilute hydrochloric acid. Warm and filter through a double paper to remove sulphur. Usually it is necessary to pour the filtrate back upon the filter several times. Add to the clear filtrate 2–3 drops of dilute ferric chloride solution. If the distillate contained hydrocyanic acid, a blood-red color will appear, or a reddish color if only traces of hydrocyanic acid are present. This is due to ferric sulphocyanate. The limit of delicacy of this test is 1:4,000,000.

Explanation.—Hydrocyanic acid and potassium hydroxide form potassium cyanide which takes sulphur from yellow ammonium sulphide and becomes potassium sulphocyanate (α). The latter in acid solution with ferric chloride forms ferric sulphocyanate (β).



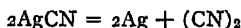
3. Vortmann's¹ Nitroprusside Test.—Add to a portion of the distillate a few drops of potassium nitrite solution; then 2–4 drops of ferric chloride solution and enough dilute sulphuric acid to give a bright yellow instead of the original yellow-brown color. Heat to boiling, add sufficient ammonium hydroxide solution to remove excess of iron and filter. Add to the filtrate 2 drops of very dilute ammonium sulphide solution. If the solution contained hydrocyanic acid, a violet color will appear and pass through blue, green and yellow. The limit of delicacy of this test is 1:312,000.

Notes.—This test is the reverse of the nitroprusside test for hydrogen sulphide and is due to conversion of hydrocyanic acid to potassium nitroprusside, $\text{K}_2\text{Fe}(\text{NO})(\text{CN})_5$, which causes the color changes when ammonium sulphide is added. Very small quantities of hydrocyanic acid give a bluish green to greenish yellow color.

¹ G. Vortmann. A New Reaction for the Detection of Small Quantities of Hydrocyanic Acid. *Monatsh. f. Chem.* 7 (1886), 416

4. Silver Nitrate Test.—Acidify a portion of distillate with dilute nitric acid and add silver nitrate solution in excess. If a white, curdy precipitate of silver cyanide (AgCN), easily soluble in ammonia, is formed, it is quite probable that the distillate contains hydrocyanic acid. The limit of delicacy of this test is 1:200,000.

The possibility of mistaking hydrochloric acid for hydrocyanic acid is excluded, if a very dilute solution was distilled, for free hydrochloric acid does not distil over under these conditions. To eliminate every trace of free hydrochloric acid that may be present, redistil the distillate once over borax. This will retain hydrochloric but not hydrocyanic acid. It is advisable to collect upon a filter, wash and dry the precipitate produced by silver nitrate solution. Silver cyanide heated in a bulb-tube forms metallic silver and cyanogen gas. With great caution note the characteristic odor of the gas, or better ignite it at the end of the bulb-tube and observe whether it burns with a purple flame. The decomposition is expressed by the following equation:



When a distillate contains hydrogen sulphide, which is the case when parts of a cadaver that has begun to putrefy are distilled, shake the precipitate produced by silver nitrate with ammonia, filter off the insoluble silver sulphide, and acidify the filtrate with dilute nitric acid. If hydrocyanic acid is present, pure white silver cyanide will now be precipitated.

5. Picric Acid Test.—Render a portion of distillate faintly alkaline with potassium hydroxide solution and warm gently with a few drops of picric acid solution. If hydrocyanic acid is present, the color of the solution will become red. This is due to formation of potassium isopurpurate.

Waller¹ mixed approximately equal volumes of distillate and an aqueous solution containing 0.05 per cent. of picric acid and 0.5 per cent. of sodium carbonate and allowed the mixture to stand for 1 hour in an incubator at 40°. In this manner he was able to prove that in poisoning by hydrocyanic acid the poison is located mainly in the heart and brain.

Notes.—This picric acid test is not specific for hydrocyanic acid, for other reducing substances such as aldehydes, acetone, hydrogen sulphide and sulphurous acid will also produce a red color with alkaline picric acid solution. For this

¹ A. D. Waller. A New Method for the Quantitative Estimation of Hydrocyanic Acid in Plant and Animal Tissues. *Proc. Roy. Soc. London* 82 (1910), 574.

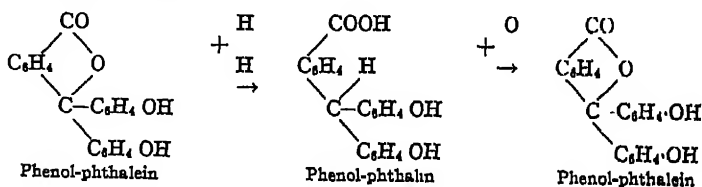
reason a negative result with this test is of greater significance than one that is positive

6. Phenol-phthalin Test.—Add a few drops of phenol-phthalin dissolved in dilute sodium hydroxide solution and then a little copper sulphate solution (1:2000) to a portion of the distillate. Even in a dilution of 1:500,000 hydrocyanic acid will produce a beautiful red color due to oxidation of phenol-phthalin to phenol-phthalein. Nitric acid, hydrogen dioxide and ferric chloride do not give this test.

Paper first moistened with alkaline phenol-phthalin solution and then with very dilute copper sulphate solution may be used. This paper turns red in air containing hydrocyanic acid.

A phenol-phthalin solution suitable for the detection of hydrocyanic acid may be prepared by boiling a solution of phenol-phthalein in dilute potassium or sodium hydroxide solution with zinc dust until the color is discharged and quickly filtering the cold liquid. This colorless, or nearly colorless filtrate, may be used for the detection of hydrocyanic acid.

Explanation.—Phenol-phthalein is reduced by nascent hydrogen to phenol-phthalin. The latter by ozone and also by hydrocyanic acid in conjunction with copper sulphate is again oxidized to phenol-phthalein which in alkaline solution produces the red color



To make the recognition of hydrocyanic acid in distillates from parts of the cadaver, or in any other material, more certain and not open to question, the author always uses the Prussian blue and sulphocyanate tests. The other tests described above are not specific for hydrocyanic acid and consequently in the forensic detection of this acid they are of subordinate significance.

Distillation of Hydrocyanic Acid in a Current of Carbon Dioxide and Quantitative Estimation

Bischoff¹ upon the basis of his results from many experiments recommends the following procedure as best adapted for the quanti-

¹ C. Bischoff. Distribution of Poisons in the Human Organism in Cases of Poisoning. Ber d Deutsch. chem. Ges 16(1883), 1337.

tative separation of hydrocyanic acid The material acidified with tartaric acid and advantageously mixed with alcohol is distilled in a current of carbon dioxide or air At the same time the distillate is caught in a concentrated solution of silver nitrate. If the distillate is not passed into such a silver solution, loss of hydrocyanic acid occurs even with the best cooling. Distillation in a current of carbon dioxide is carried on in the apparatus shown below (Fig. 10)

Introduce the comminuted material with a small quantity of water into the distilling flask (a) provided with a two-hole stopper, one of which extends almost to the bottom of the flask and the other is in connection with a Liebig condenser (b) A carbon dioxide generator (c) is connected with a wash-bottle The receiver is an Erlenmeyer flask, containing a few cc of

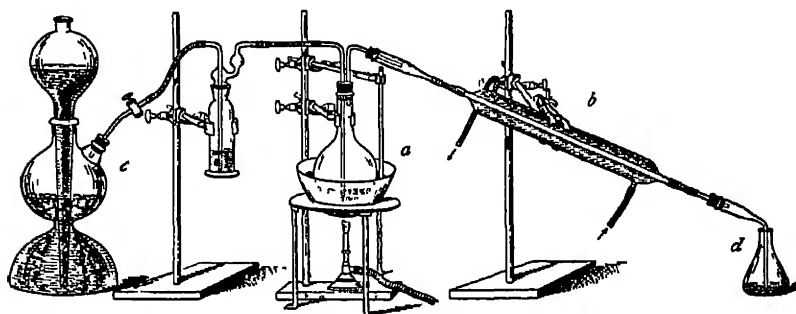


FIG. 10 —Apparatus for distillation of hydrocyanic acid in a current of carbon dioxide

water into which the end of the adapter (d) dips. It is advisable to set the receiver in ice-water.

In quantitative estimations take a weighed quantity of the well-mixed and comminuted material and instead of water put into the Erlenmeyer flask a few cc. of saturated silver nitrate solution, allowing the end of the adapter to dip below the surface. When the apparatus is ready, acidify the contents of the flask with tartaric acid, start the carbon dioxide, and gently warm the flask in a water-bath or oil-bath. A quantitative estimation takes 2–3 hours and at the end of that time all hydrocyanic acid will be in the receiver. Collect the precipitate of silver cyanide upon a filter, wash it with water until free from silver, dry in a weighed porcelain crucible and ignite. From the weight of metallic silver obtained calculate the amount of hydrocyanic acid by multiplying by 0.2505 ($\text{Ag}:\text{HCN} = 107.8:27 = 0.2505$).

If an oil-bath is used in a qualitative test for hydrocyanic acid, one and the same portion of material, after the first fraction obtained by gentle warming is tested for hydrocyanic acid, may be used for the detection of less volatile substances such as phenols, chloral hydrate, formaldehyde, aniline and carbon disulphide, by bringing the contents of the flask to an active boil.

Detection of Hydrocyanic Acid in Presence of Potassium Ferrocyanide

When material contains non-toxic potassium ferrocyanide, hydrocyanic acid will appear in the distillate from a solution acidified with tartaric acid. In an experiment where 1 per cent potassium ferrocyanide solution was distilled with 0.03 gram of tartaric acid, the distillate contained considerable hydrocyanic acid. Carbon dioxide passed into hot, aqueous potassium ferrocyanide solution will liberate hydrocyanic acid even at water-bath temperature (75°). To test for potassium ferrocyanide beforehand, shake some of the original material with water and filter. Test the filtrate with ferric chloride solution and dilute hydrochloric acid. If there is a precipitate of Prussian blue, potassium ferrocyanide is present. To detect free hydrocyanic acid, potassium or sodium cyanide, but not hydrocyanic acid from mercuric cyanide, add to the material acid sodium carbonate in not too small quantity and distil. Even long distillation over a free flame by this method will liberate hydrocyanic acid only from simple cyanides and not from potassium ferrocyanide! (W. Autenrieth¹)

Detection of Mercuric Cyanide

When an aqueous solution of mercuric cyanide, which is exceedingly poisonous, is distilled with tartaric acid, the distillate will contain hydrocyanic acid only when a large quantity of mercuric cyanide is present. Distillation of 100 cc. of 1 per cent aqueous mercuric cyanide solution yields a distillate that gives the Prussian blue test distinctly. But if the quantity of mercuric cyanide is less and the solution very dilute (for example, 100 cc. of 0.01 per cent solution), there will not be a trace of hydrocyanic acid in the distillate, even though the solution is strongly acidified with tartaric acid. If, however, a few cc. of freshly prepared hydrogen sulphide water are added and distillation is resumed, mercuric cyanide will be completely decomposed and the distillate will contain hydrocyanic acid.

Detection of Mercuric Cyanide in Presence of Potassium Ferrocyanide

The method of detecting hydrocyanic acid from simple cyanides, in presence of potassium ferrocyanide, is not applicable to mercuric cyanide. Long distillation, even from saturated acid sodium carbonate solution, gives no trace of hydrocyanic acid. But distillation in presence of not too little acid sodium carbonate, after addition of a few cc. of freshly prepared, saturated hydrogen sulphide solution, liberates hydrocyanic acid from mercuric cyanide but not from potassium

¹ W. Autenrieth: Contribution to the Knowledge of Yellow Prussiate of Potash and the Detection of Hydrocyanic Acid in Presence of Ferrocyanides. Arch. d. Pharmazie 231 (1893), 99

ferrocyanide It is possible to detect CN^- -ion by this method, when very little mercuric cyanide is mixed with considerable potassium ferrocyanide. For example, 0.01 gram of mercuric cyanide in 100 cc of 10 per cent potassium ferrocyanide solution can be detected. If potassium ferrocyanide is distilled direct with hydrogen sulphide without addition of acid sodium carbonate, the distillate will contain considerable hydrocyanic acid.

Formation of Hydrocyanic Acid from Normal Organic Substances of Animal Organism at Higher Temperature and in Oxidation

Sulphocyanic acid, $HSCN$, occurring normally in the human body, is said to undergo decomposition into hydrocyanic acid and persulphocyanic acid, but only in concentrated aqueous solution in absence of mineral acids, or in presence of small quantities of other acids. In presence of potassium sulphocyanate Ganassini¹ has subjected organic substances to distillation, after adding tartaric or sulphuric acid, and been unable to detect hydrocyanic acid in the distillate. In presence of much potassium sulphocyanate, however, the distillate contained traces of free sulphocyanic acid. During distillation this acid as a rule is decomposed in the following manner



Consequently in toxicological investigations errors can not arise in this manner on account of the presence of sulphocyanic acid.

Ganassini, however, maintains that he has positively detected hydrocyanic acid in distillates from organs of healthy animals and that this acid was not present beforehand. Consequently this hydrocyanic acid must have come into existence during distillation! Ganassini assumes that these traces of hydrocyanic acid arise as a result of decomposition of protein substances and xanthine bases of these organs taking place at higher temperatures. Over-heating of these substances is probably brought about when through coagulation they become firmly attached to the walls of the distilling flask, dried and finally brought to the temperature of the distilling flask. Haematin in fact at 200° underwent profound decomposition, yielding hydrocyanic acid together with other substances. To eliminate this source of error, distillation should not be conducted over a free flame but in an oil-bath, the temperature of which does not exceed 120° . In such distillations the author has for years used a bath of paraffine oil. He has never been able, however, in distilling parts of organs and blood over a free flame upon an asbestos wire-gauze to establish any formation of hydrocyanic acid, provided the material did not contain this acid at the start.

Plummer² has made the observation, which deserves mention but is of no practical significance in forensic chemistry, that hydrocyanic acid is formed from protein substances as a result of oxidation. Upon oxidation with a mixture of equal volumes of water, concentrated nitric and sulphuric acid, casein upon the average gave 0.74, haemoglobin 0.56, fibrin 0.66, Witte's peptone 0.53, egg albumin 0.60 and gelatin 0.2 per cent. of hydrocyanic acid. Casein gave the

¹ D. Ganassini. *Bull. Chm. Farm.* 44, 508 and 519.

² R. H. Aders Plummer: Formation of Hydrocyanic Acid in the Oxidation of Protein Substances. *Journal of Physiology* 31 (1904), 65

same quantity of hydrocyanic acid when oxidized with potassium dichromate and sulphuric acid but only small quantities when oxidized in sulphuric acid solution with potassium permanganate. If protein substances before oxidation are first hydrolyzed with dilute sulphuric acid, they also give the same quantities of hydrocyanic acid mentioned above. The amino-acids, glycocoll, alanine, α -pyrrolidine-carboxylic acid, aspartic acid, as well as guanidine and succinimide, oxidized with nitric-sulphuric acid gave traces of hydrocyanic acid, at most 0 per cent, and tyrosine alone 0.79 per cent.

Relative Stability of Hydrocyanic Acid in Cadaveric Putrefaction

According to L. Lewin the length of time during which hydrocyanic acid can be detected in the animal organism depends mainly upon the degree of putrefaction and the quantity of poison that has been taken. As a matter of fact when putrefaction has set in quite actively, the poison very quickly disappears from the cadaver. In view of these statements a case described by W. Autenrieth¹ is of interest. Weighable quantities of hydrocyanic acid were obtained from the internal organs taken from the exhumed body of a five year old child who had been in the ground for 45 days. These particular internal organs (liver, spleen, kidneys and heart) after this long period were surprisingly well preserved and without any of the strong odor characteristic of decomposition. The putrefactive experiments made by Autenrieth (loc. cit.) with parts of organs and blood, to which bitter almond water or potassium cyanide had been added, are also in agreement with this statement. The results of these experiments showed that hydrocyanic acid in contact with parts of animal organs undergoes decomposition is partially destroyed. This decomposition, however, requires very long time to come to completion, for even after 60 days there was still present in the mixtures that were in a high state of putrefaction more than 4 per cent. of the hydrocyanic acid originally added. The concentration of the hydrocyanic acid added to these putrefying mixtures apparently has an influence upon the quantity of the acid remaining unchanged, for hydrocyanic acid has strong toxic action upon ferments and bacteria. In accordance with this view processes of putrefaction begin more slowly in a 1:10,000 concentration of hydrocyanic acid and proceed less actively than in the lower concentration of 1:20,000. Addition of grape sugar to the putrefying mixtures had no considerable influence upon the quantity of hydrocyanic acid destroyed or combined, not even when the quantity of sugar added was ten times greater than that normally present in human blood.

Cram² has reported a fatal case of poisoning caused by whiskey containing potassium cyanide. Five days after death 0.053 gram of potassium cyanide was found in the stomach-contents, this quantity being calculated for the total contents. There was still 0.039 gram of cyanide present 25 days after death and not until 76 days had elapsed could no trace of the poison be detected. In the viscera:

¹W. Autenrieth: Detection of Hydrocyanic Acid in an Exhumed Body and the Relative Stability of Hydrocyanic Acid during Putrefaction. *Ber. d. Deutsch. Pharm. Ges.* 20 (1910), 432.

²M. P. Cram: A Case of Potassium Cyanide Poisoning. *Journ. Amer. Chem. Soc.* 36 (1914), 606.

on the other hand, a short time after death not a trace of hydrocyanic acid could be detected

According to Jollyman¹ also, the stability of potassium cyanide in the stomach after death is far greater than is generally supposed. Even after six months the stomach contents of a negro, who had died from potassium cyanide poisoning, gave distinct tests for hydrocyanic acid. In another experiment Jollyman obtained 0.5 gram of potassium cyanide from a hog weighing 70 kilograms, since the animal did not die, it was shot 15 hours after the experiment began. Seven weeks later hydrocyanic acid could still be distinctly recognized in the stomach of the same hog.

Hydrocyanic Acid Poisoning; Symptoms and Lethal Dose

In the case of man, poisoning from hydrocyanic acid, potassium or sodium cyanide, may have a fatal termination very quickly, almost immediately after the poison has been taken, or death may not ensue until several hours have elapsed. Consequently the symptoms exhibited in a poisoning of this kind may be extremely varied. In the first type of poisoning, that is, when a considerable quantity of hydrocyanic acid or of a poisonous metallic cyanide has been taken in one dose, the person poisoned drops unconscious at once after taking the poison and the pupils are greatly enlarged. He takes at most but a few breaths and dies almost immediately. When the poison is taken internally in doses that are not so large but still sufficient to cause death, the symptoms that appear are dizziness, impairment of the mental faculties, headache, dimness of the field of vision, rush of blood to the head, uneasiness, oppression, pains in the chest and palpitation of the heart. At a more advanced stage of poisoning, there appear impairment of respiration, total unconsciousness and violent convulsions. Finally respiration ceases entirely, whereas the heart continues to beat to the very end (R. Kobert).

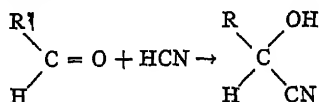
The lethal dose of anhydrous hydrocyanic acid is placed at 0.06 gram for an adult and that of pure potassium cyanide at 0.15 gram. The lethal dose of commercial potassium cyanide, which frequently contains quite considerable quantities of potassium cyanate and carbonate, is placed at 0.25–0.35 gram and that of bitter almond water and cherry laurel water at about 60 grams. The fact that 0.24 gram of commercial potassium cyanide taken internally and 0.3 gram as an enema have caused the death of adults is in accord with the generally accepted view.

Absorption and Toxic Action of Hydrocyanic Acid

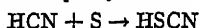
In whatever way applied, hydrocyanic acid is easily absorbed, even from the skin. So rapid is the absorption of this poison that there is evidence of an intoxication after a few seconds, or a few minutes at most. This is especially true when a fatal dose of anhydrous hydrocyanic acid is given internally to a man or animal. Part of the poison thus absorbed passes from the body unchanged by way of the lungs. Another part, usually much less, is eliminated by the kidneys and passes into the urine. Sweat also is said to contain hydrocyanic

¹ W. H. Jollyman: The Stability of Potassium Cyanide in the Stomach after Death. *Chemiker-Zeitung* Jahrgg. 29, 350.

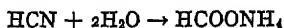
acid Otherwise, views with regard to the fate of hydrocyanic acid in the animal organism vary widely Thus it is assumed that hydrocyanic acid takes part in the formation of cyanohydrins with the dextrose of the blood and with other carbohydrates of the animal organism.



and that for this reason it can no longer be detected in the cadaver by means of the usual chemical reactions The assumption also that hydrocyanic acid combines direct with the proteins of the body or with the loosely bound sulphur of proteins to form derivatives of sulphocyanic acid



is suggested as possible Finally, hydrocyanic acid may also be oxidized, or hydrolyzed through the agency of enzymes and converted into ammonium formate



All of these hypotheses, regarded from the purely chemical standpoint, are more or less plausible In addition, it will be shown below that in fatal poisoning a not inconsiderable part of the hydrocyanic acid can still be found as such in the human body and even in the exhumed cadaver.

Hydrocyanic acid acts as a poison for animal and plant protoplasm. For example, it makes impossible the germination of seeds of various plants, although not permanently, but suspends it as long as the poison is present. That is, hydrocyanic acid paralyzes plant enzymes that take part in the germination of seeds. It does not paralyze the assimilating but the dissimilating enzymes. The effect of hydrocyanic acid upon human metabolism is quite similar. It interferes particularly with the action of that enzyme which causes transfer of oxygen from blood-corpuscles and thereby gives rise to oxidative processes (oxidation ferment) of the animal body Geppert has shown by very careful experiments in metabolism that warm-blooded animals under the influence of hydrocyanic acid take up less than the normal amount of oxygen and consequently give off less carbon dioxide, even though relatively large quantities of oxygen are administered artificially. Consequently the toxic action of hydrocyanic acid depends upon an internal asphyxiation of the organs in presence of an excess of oxygen The oxidative processes of the blood are checked and so little oxygen is used that the venous blood becomes arterial, that is, contains a large quantity of oxyhaemoglobin As a result the color of the venous blood is bright red The occurrence of lactic acid in the blood and a decrease in its alkalinity are also attributable to the severely disturbed oxidative processes of animal metabolism brought about under the influence of hydrocyanic acid. The processes of normal metabolism cause complete oxidation of lactic acid to carbon dioxide and water. Consequently the appearance of lactic acid in the blood of warm-blooded animals is at most transitory and it is not found in the urine at all As a result of very deficient oxidation in the animal organism during hydrocyanic acid poisoning, not only lactic acid but not infrequently dextrose appears in the urine

Autopsy in Hydrocyanic Acid Poisoning

The blood and all the body cavities have a distinct odor like that of hydrocyanic acid. If some time has elapsed since death, the odor of hydrocyanic acid in the blood and in the abdominal cavity may not be noticeable. In such cases, however, it can frequently still be distinctly recognized in the cranial cavity, at least as soon as it is opened. The blood as a result of the change of venous to arterial blood has a striking bright red color. Moreover there is only a little coagulum in hydrocyanic acid blood and that is loose. For this reason, it usually remains fluid and mobile for a considerable time. This behavior of hydrocyanic acid blood is to be attributed to the fact that this acid in the capacity of an enzyme poison has more or less checked the enzymatic process of fibrin formation. In hydrocyanic acid poisoning the autopsy shows further that the blood-vessels of the brain and the right heart are usually entirely full. The lungs, liver and kidneys may also show that the blood-vessels are very full of blood. The bronchial tubes may be reddened and filled with reddish froth. In poisonings from free hydrocyanic acid the gastric mucosa, except for some slight extravasation of blood, may appear unchanged. In the case of potassium and sodium cyanide, the action of caustic alkalies is noticeable, for these salts are considerably hydrolyzed.



The color of the death-spots is more or less characteristic of hydrocyanic acid poisoning. In more than 30 per cent of the cases of hydrocyanic acid poisoning, they do not have a dark but a striking bright red color that would at once be noticed even by a novice.

PHENOLS

General Behavior and Reactions

All phenols dissolve in aqueous solutions of alkaline hydroxides forming phenolates:



and acids, even carbon dioxide, reprecipitate them from these solutions:



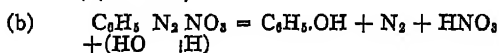
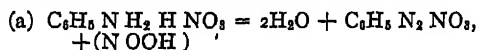
This solubility in solutions of alkaline hydroxides is due to the presence of one or more hydroxyl-groups in the benzene ring. Most of the monacid phenols are volatile with steam. For this reason, they can be separated from other materials by distillation with water and they then impart to the distillate their more or less specific odor. Belonging to this class of phenols, are such substances as carbolic acid, the cresols, guaiacol and creosol, thymol, as well as α - and β -naphthol.

On the other hand, phenols containing more than one hydroxyl group are practically non-volatile with steam and as a rule without characteristic odor. Substances of more importance normally belong to this group, such as catechol, resorcinol, quinol, pyrogallol.

General Phenol Reactions

1. Millon's Test.—This is also known as Plugge's¹ reaction. To a few drops of Millon's reagent, the aqueous solution of a monophenol even in the cold, or when warmed if only traces of the phenol are present, takes on a light to dark red color. This test seems to be specific for monacid phenols and their derivatives. Consequently salicylic acid, p-oxy-benzoic acid, tyrosine, salicylic alcohol, salicylic aldehyde also give Millon's test. In the case of the ethers of phenols this test is not given until later.

This reaction takes place when one or more hydrogen atoms of the benzene ring of the monacid phenols are replaced by hydrocarbon radicals. But substituents as chlorine, bromine, iodine or carbon-free radicals prevent it. The fact that aniline and other primary, aromatic amines give this test is due to the presence in the reagent of free nitric and nitrous acid. They first form diamine salts and these warmed with water give phenols.



Phenols containing more than one hydroxyl-group do not give the typical red color but some of them reduce Millon's reagent with separation of mercury.

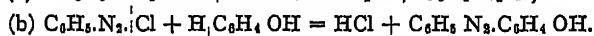
2. Ferric Chloride Test.—Dilute ferric chloride solution imparts a characteristic color to the aqueous solution of many phenols and their derivatives. These colors are blue, blue-violet, red-violet, red and green. But aqueous solutions of substances containing free phenolic hydroxyl-group, such as antipyrine and pyrazolone, also give colors with ferric chloride. In the case of many phenols such as quinol and the two naphthols, ferric chloride has an oxidizing action. Free dilute hydrochloric, or sulphuric acid, discharges the colors produced by ferric chloride in aqueous solutions of phenols.

¹ P. C. Plugge: Mercuric Nitrate containing Nitrous Acid as a Reagent for Aromatic Compounds containing a Hydroxyl-group in the Benzene Ring. *Archiv. d. Pharmazie.* 228 (1890), 9.

3. **Liebermann's¹ Test.**—Dissolve a small quantity of a phenol in concentrated sulphuric acid and add a few drops of a solution containing 0.6 gram of potassium nitrite in 10 grams of concentrated sulphuric acid (Liebermann's reagent). Various phenols give different colors that change upon addition of glacial acetic acid, or water, and upon saturation with ammonia. Carboic acid gives a blue solution that becomes red upon addition of glacial acetic acid, or water, and turns blue again upon saturation with ammonia. Liebermann's test is due to the formation of a colored nitroso-derivative.

4. **Eykman's² Test.**—This is a modification of Liebermann's reaction. Add to a dilute aqueous solution of a phenol a few drops of an alcoholic solution of ethyl nitrite (*Spiritus Aetheris Nitrosi*) and then concentrated sulphuric acid from a pipette so that it forms a distinct under-layer. A red zone will appear at the contact surface of the two liquids. Various colors appear upon addition of glacial acetic acid, or water, and excess of ammonia. In a dilution of 1:200,000 carboic acid still gives a distinct red color that changes to blue upon addition of ammonia.

5. **Azo-Dye Formation.**—Diazonium salts, such as diazo-benzene chloride, obtained by adding sodium nitrite to a hydrochloric acid solution of aniline hydrochloride, couple with phenols and naphthol forming oxyazo-dyestuffs:



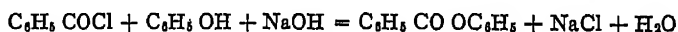
Dissolve 2-3 drops of aniline in an excess of dilute hydrochloric acid, cool with ice to 0°. First add to this solution a few drops of a 5 per cent. sodium nitrite solution, then the faintly alkaline phenol solution, and finally an excess of sodium hydroxide solution. Yellow, scarlet or bordeaux-red colors appear, or colored precipitates consisting of the azo-dyestuff formed. Under the same conditions many aldehydes and ketones also give more or less pronounced colorations.

6. **Schotten-Baumann Benzoylation Test.**—In comparison with the general phenol color-reactions already mentioned, this test possesses the advantage, not to be undervalued in forensic chemistry, of giving even with very dilute aqueous phenol solutions well-defined

¹ C. Liebermann, *Dyestuffs formed from Aromatic Oxy-Compounds and Nitrous Acid*. *Ber. d. Deutsch. chem. Ges.* 7 (1874), 1098.

² Eykman, *Zeitschr. f. analyt. Chemie* 22 (1883), 576.

substances that usually crystallize well and melt sharply. By a determination of the melting-point the substance can be more definitely characterized. Add to the given aqueous phenol solution about 10 cc. of sodium hydroxide solution (10 per cent.) and also 1-2 cc of benzoyl chloride. Shake the mixture vigorously until the odor of benzoyl chloride has disappeared. Throughout the entire test the reaction should be alkaline. If it becomes acid, more sodium hydroxide solution must be added. The ester of benzoic acid that is formed usually separates as a white, crumbly mass insoluble in water. Filter it off and wash with cold water until free from alkali. Frequently it can be crystallized from alcohol or acetone by preparing a saturated solution of the ester at ordinary temperature, mixing with about the same volume of water and setting in ice:



Primary and secondary amines, as well as ammonia, which can also be benzoylated, should not be present. Should the liquid under examination contain such compounds, it must be strongly acidified with dilute sulphuric acid and then distilled. The resulting distillate will contain only phenols. Even from a solution containing an excess of sulphuric acid, traces of aniline will pass over into the distillate. Alcohols usually give liquid benzoyl derivatives. The benzoyl derivatives of the more common phenols and naphthols are the following:

Name	Formula	Crystalline Form	Melting-Point
Phenyl Benzoate	$\text{C}_6\text{H}_5\text{COOC}_6\text{H}_5$	Prisms	68-69°
p-Cresyl Benzoate	$\text{C}_6\text{H}_5\text{COOC}_6\text{H}_4\text{CH}_3$	Plates	71.5°
α -Naphthyl Benzoate	$\text{C}_6\text{H}_5\text{COOC}_{10}\text{H}_7$	Plates or Prisms	56°
β -Naphthyl Benzoate	$\text{C}_6\text{H}_5\text{COOC}_{10}\text{H}_7$	Plates or Prisms	108°

These esters can also be recrystallized from an alcohol-ether mixture.

Supplement to Volatile Phenols

(Maltol¹)

Maltol, $\text{C}_8\text{H}_6\text{O}_3$, is formed in the preparation of caramel from malt, possibly from maltose or isomaltose. Ether or chloroform extracts this substance from the condensed vapors given off during caramelization and also from beer-wort. Maltol crystallizes in monoclinic prisms and plates from a cold, saturated solution in 50 per cent alcohol. Chloroform gives more compact crystals. Maltol dissolves with difficulty in cold water or benzene; more readily in hot water, alcohol, ether or chloroform; and is insoluble in petroleum ether. It dissolves in caustic alkaline solutions but is reprecipitated by carbon dioxide. Maltol sublimes in shining leaflets but is reprecipitated by carbon dioxide.

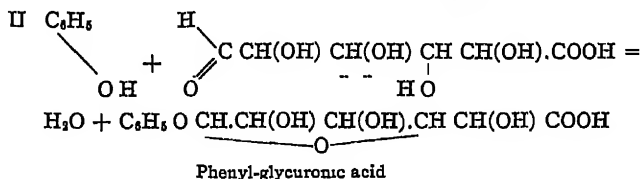
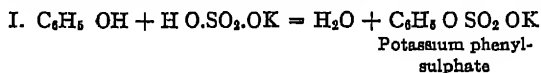
¹ J. Brand Maltol Ber. d. Deutsch. chem. Ges. 27 (1894), 806; K. Killiani and M. Bazlen: Maltol Ber. d. Deutsch. chem. Ges. 27 (1894), 3115.

It reduces silver solution in the cold and Fehling's solution with heat. Mention should be made of the fact that the aqueous solution of maltol, although this compound is not a phenol, gives an intense violet color with ferric chloride solution. In distinction from the phenols volatile with steam and salicylic acid, a maltol solution does not turn red when warmed with Millon's reagent.

Since maltol gives a mono-benzoyl derivative when shaken with benzoyl chloride and sodium hydroxide solution, its molecule must contain one hydroxyl-group.

Physiological Behavior of Phenols

The phenols also show quite a close resemblance in their behavior in the animal organism in that the latter seeks to get rid of the toxic effects of phenols by quickly eliminating them again after they have been administered and absorbed. Conjugation with sulphuric acid (I) as well as with glycuronic acid (II) is the method followed in getting rid of the poisons. In the form of their alkali salts the conjugated acids thus formed are eliminated in the urine through the kidneys.



As a result of the formation of "conjugated sulphuric acid," which is present in small quantity in every normal human urine, the "sulphate-sulphuric acid," also normally present, in part disappears. Consequently in a poisoning caused by a phenol the conjugated sulphuric acid, known also as ethereal sulphuric acid, is more or less strongly increased. On the other hand, the quantity of sulphate-sulphuric acid in the urine, as compared with that normally eliminated, is diminished. A urine containing conjugated glycuronic acids is laevo-rotatory and reduces Fehling's solution.

In the animal organism monacid phenols are oxidized in part to di-acid phenols. As a consequence "carbolic urine" eliminated, following administration of carbolic acid, contains quinol-sulphuric acid in addition to phenyl-sulphuric and phenyl-glycuronic acid. As a result of severe impairment of the kidneys the sediment of "carbolic urine" frequently contains albumin, blood-pigment, free blood-corpuscles, epithelial and cylindrical cells.

CARBOLIC ACID

Carbolic acid, or benzo-phenol, $\text{C}_6\text{H}_5\text{OH}$, forms long, colorless, pointed crystals, or a white, radiating crystalline mass having a characteristic odor. Liquid carbolic acid (Acidum Carbolicum Liquefactum) contains 10 per cent of water and is a clear, colorless

liquid, or has a faint reddish color. On the other hand, carbolic acid dissolves in about 20 parts of water and further is readily soluble in alcohol, ether, and chloroform as well as in solutions of caustic alkalis. Pure anhydrous carbolic acid melts at $40-42^{\circ}$ and boils at $178-182^{\circ}$.

Carbolic acid distills relatively easily with steam, but rather prolonged distillation is required to get the last portions over. In most cases it may be recognized by its characteristic odor. When present in larger quantities, colorless or reddish oil-drops usually float about in the milky distillate. These dissolve in potassium or sodium hydroxide solution and the distillate becomes clear. Since small quantities of volatile phenols, especially of p-cresol, are formed during putrefaction of proteins, distillates from parts of cadavers that have passed into a high state of putrefaction almost always contain traces of these two phenols. Such a distillate from putrefied cadaveric material consequently gives Millon's test and usually also the test with bromine water.

The following tests may be used for the purpose of detecting carbolic acid.

1. Millon's Test.—Millon's reagent,¹ heated with a solution containing only a trace of carbolic acid, produces a red color. An

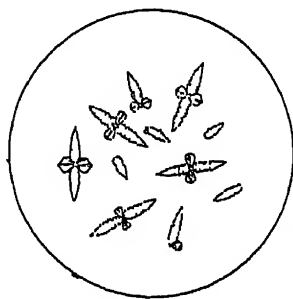


FIG. 11.—Tribromo-phenyl hypobromite crystals from a dilution of 1:20,000.

aqueous solution containing only 20 milligrams of carbolic acid, diluted 1:100,000, will give a distinct red color. If the phenol solution is not very dilute, the color will appear even in the cold. Though a very delicate test, it is not characteristic of carbolic acid, because very many aromatic substances, especially other monacid phenols and their derivatives resemble carbolic acid in their behavior toward Millon's reagent.²

2. Bromine Water Test.—Excess of bromine water produces a yellowish white, crystalline precipitate, consisting really of tribromo-phenyl

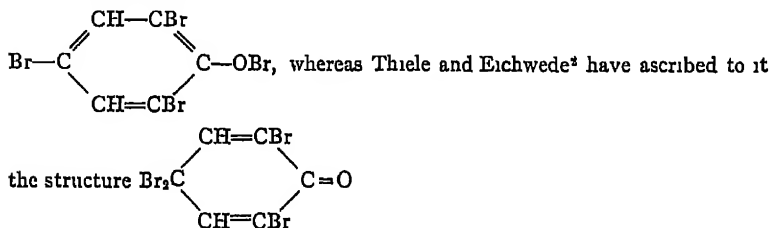
¹ Para-hydroxy-phenyl-acetic acid and hydro-para-cumaric acid are formed in the putrefaction of proteins but are not volatile with steam. Consequently they cannot be present in a distillate that is to be tested for volatile phenols.

² Traces of salicylic acid volatilize with steam, at least in such quantity that it can be detected in the distillate with Millon's reagent.

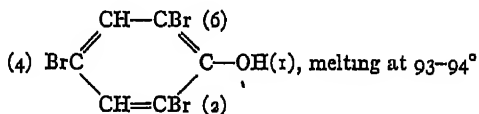
hypobromite, even with very dilute carbolic acid solutions. It is a very delicate test for carbolic acid. Phenol diluted 1:50,000 yields after some time a precipitate made up in part of well-formed crystals (Fig. 11). Pure tribromo-phenyl hypobromite melts at $131-133^{\circ}$, giving off bromine at the same time.

Salicylic alcohol (saligenin), salicylic aldehyde, salicylic acid, and p-hydroxybenzoic acid are also converted quantitatively by an excess of saturated bromine water even in the cold into tribromo-phenyl hypobromite.

If there is a sufficient excess of bromine water to give the supernatant liquid a brownish red color, the precipitate consists only of tribromo-phenyl hypobromite, $C_6H_2Br_3OBr$. Benedikt¹ regards this compound as a bromo-phenoxy-tribromobenzene having the structure



This reaction takes place so easily that carbolic acid may even be determined quantitatively as this tetrabromo-derivative (see page 47). It melts at $132-134^{\circ}$ with evolution of bromine and crystallizes in lemon-yellow leaflets from alcohol-free chloroform or ligroin. Heated with alcohol, acetone, xylene, or aqueous sulphurous acid, this compound loses bromine and changes at once to 2, 4, 6-tribromo-phenol:



3. Ferric Chloride Test.—Very dilute ferric chloride solution, added drop by drop, imparts a blue-violet or blue color to aqueous carbolic acid solutions. Addition of dilute hydrochloric, or sulphuric acid, changes this color to yellow. The color also disappears upon addition of alcohol (distinction from salicylic acid). This test is not as delicate as 1 and 2. It is entirely negative in presence of mineral acids. The limit of delicacy is about 1:1000.

¹ R. Benedikt: Bromoxy-Derivatives of Benzene. *Annalen d. Chemie* 199 (1879), 127.

² J. Thiele and H. Eichwede: The Constitution of Tribromo-phenyl Hypobromite. *Ber. d. Deutsch. chem. Ges.* 33 (1900), 673.

4. **Hypochlorite Test.**—Add a few cc. of ammonium hydroxide solution to a dilute, aqueous carbolic acid solution, and then 2-4 drops of freshly prepared calcium or sodium hypochlorite solution. Gentle warming will produce a blue color. Very dilute carbolic acid solutions after some time give only a green to blue-green color. Flückiger¹ allows bromine vapor to come in contact with the phenol solution that has been mixed with a little ammonium hydroxide solution in a porcelain dish. Ortho- and meta-cresol also give a blue color but that given by para-cresol is a dirty brownish green. This test is also known as Lex's reaction.

5. **Melzer's Benzaldehyde Test.**²—Add 2 cc. of concentrated sulphuric acid to 1 cc. of the solution (distillate) to be tested for carbolic acid, then 1-2 drops of benzaldehyde and heat. The liquid that is yellowish brown in the cold takes on a dark red color in presence of phenol. At the same time a red resinous substance appears unless the solution is too dilute. When cold, add 10 cc. of water and enough potassium hydroxide solution to give a distinct alkaline reaction. If carbolic acid is present, a beautiful violet-blue color will appear. To obtain this coloring-matter, acidify the solution, extract with ether and evaporate the solvent. Alkalies added to alcoholic solutions of the coloring-matter produce a blue color that acids discharge. This is a very delicate test. One cc. of 0.5 per cent. carbolic acid solution (= 0.0005 gram of carbolic acid) will still give a distinct blue color. Of the three cresols only o-cresol gives this test.

6. **Formaldehyde Test.**—Pouget³ recommends a mixture of 10 cc. of concentrated sulphuric acid, 10 cc. of water, and 20 drops of 40 per cent. formaldehyde solution. This reagent for phenols is used more advantageously as two separate liquids, one of which (No. 1) consists of a mixture of 10 cc. of water and 20 drops of formaldehyde solution and the other (No. 2) of concentrated sulphuric acid. Equal volumes of these two liquids are mixed as required. With phenols, and substances containing at least one phenolic-group, this reagent gives characteristic precipitates or colors. Put about 0.02 gram of the substance in a test-tube and then add 1 cc. of solution No. 1 and 1 cc. of solution No. 2. If the given substance is a liquid, add 1 cc. to 1 cc. of concentrated sulphuric acid and then 2 drops of formaldehyde solution.

¹ Pharmazeutische Chemie 1879, page 287.

² H. Melzer: Contributions to Forensic Chemistry. Zeitschr. f. analyt. Chemie 37 (1898), 345.

³ J. Pouget. A General Reagent for Phenols. Bull. de Sciences Pharm. 16 (1900), 142, Schweiz. Wochenschr. f. Chem. u. Pharm. 350 (1909).

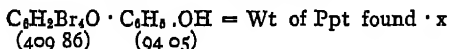
QUANTITATIVE ESTIMATIONS OF PHENOL

1. Gravimetric Estimation as Tribromo-phenyl Hypobromite

(Autenrieth and Beuttel¹)

This method is based upon the behavior of aqueous phenol solutions toward an excess of saturated bromine water which completely precipitates the phenol as tribromo-phenyl hypobromite, $C_6H_2Br_3OBr$ (see above). Since the latter is practically insoluble in cold bromine water, this method of estimating carbohc acid gives very satisfactory results.

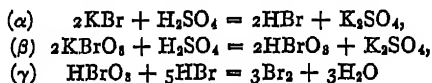
Procedure.—Place the aqueous phenol solution in a large glass-stoppered flask. Add gradually, while shaking, saturated bromine water until the supernatant liquid has a red-brown color and bromine vapor is visible above the solution. Shake frequently and allow to stand for 2-4 hours. Then collect the precipitate in a weighed Gooch crucible and dry in a vacuum desiccator (Fig. 13, see page 96) over sulphuric acid to constant weight. On the basis of the following proportion calculate the weight of phenol corresponding to the weight of the precipitate:



Since the ratio $94.05/409.86 = 0.2295$, the weight of phenol may be found by multiplying the weight of the precipitate by 0.2295

2. Beckurts-Koppeschaar² Volumetric Method

Dilute sulphuric acid liberates hydrobromic acid from potassium bromide (α) and bromic acid from potassium bromate (β). These two acids react according to (γ) with liberation of bromine:

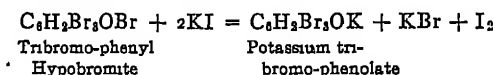


Therefore addition of dilute sulphuric acid to a mixture of potassium bromide and bromate solutions liberates bromine that will

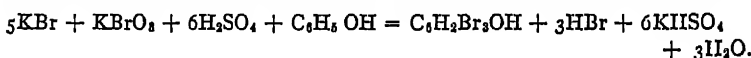
¹ W. Autenrieth and Fr. Beuttel. Estimation of Phenol, Salicylic Alcohol, Salicylic Acid, Etc. as Tribromo-phenyl Hypobromite Arch d Pharm. 248 (1910), 112

² H. Beckurts Quantitative Estimation of Carbohc Acid as Tribromo-phenol. Arch. d. Pharm. 224 (1886), 561-572 W. F. Koppeschaar: Volumetric Estimation of Phenol. Zeitschr. f. analyt. Chemie 15 (1876), 232.

convert phenol into a mixture of tribromo-phenol and tribromo-phenyl hypobromite. The excess of free bromine, as well as the one labile bromine atom of tribromo-phenyl hypobromite, will displace iodine from potassium iodide and finally all the phenol will be present as tribromo-phenol



One molecule of phenol requires 6 atoms of bromine, as shown in the equation:



Titration requires the following standard solutions

1. 0.01 n-potassium bromide solution, containing $\frac{5\text{KBr grams}}{100} = \frac{595.6}{100} = 5.956$ grams of KBr in 1000 cc
2. 0.01 n-potassium bromate solution, containing $\frac{1\text{KBrO}_3}{100} = \frac{167.17}{100} = 1.6717$ grams of KBrO₃ in 1000 cc
3. 0.1 n-sodium thiosulphate solution, containing 0.1 Na₂S₂O₃ 5H₂O grams = 24.83 grams in 1000 cc
4. Potassium iodide solution, containing 125 grams of KI in 1000 cc

Procedure.—Put about 25 cc. of aqueous phenol solution (distillate) into a flask having a tight glass stopper. Add 50 cc. each of 0.01 n-potassium bromide and 0.01 n-potassium bromate solutions, then 5 cc. of pure concentrated sulphuric acid and shake vigorously for several minutes. The gradually increasing opalescence of the solution becomes more and more marked, as tribromo-phenol and tribromo-phenyl hypobromite are precipitated. The yellow color that soon appears shows excess of bromine. Open the flask after 15 minutes, add 10 cc. of potassium iodide solution, shake and titrate free iodine after a few minutes with 0.1 n-sodium thiosulphate solution

Calculation.— $\frac{6 \text{ gram-atoms Br}}{100} = \frac{6 \times 79.96}{100} = 4.7976$ grams of bromine are set free from a mixture of 1000 cc each of 0.01 n-potassium bromide and 0.01 n-potassium bromate solution. A mixture therefore of 50 cc. each of the two solutions will give 0.23988 gram of bromine. This quantity of bromine can convert 0.04704 gram of phenol into tribromo-phenol.

$$\begin{array}{ccc} 6\text{Br} & \text{C}_6\text{H}_5\text{OH} & \\ 479.76 & 94.05 & = 0.23988 \cdot x \quad (x = 0.04704) \end{array}$$

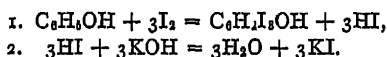
1 cc of 0.1 n-sodium thiosulphate solution corresponds to 0.012697 gram of iodine and this quantity of iodine to 0.007996 gram of bromine. But 0.007996 gram of bromine will convert 0.00157 gram of phenol into tribromo-phenol:

$$\begin{array}{rcl} 6\text{Br} & \text{C}_6\text{H}_5\text{OH} & \\ 479.76 & 94.05 & = 0.007996 \cdot x \quad (x = 0.00157) \end{array}$$

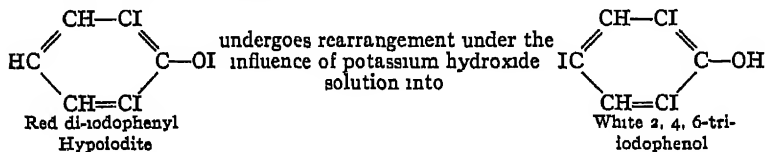
Consequently, for each cc. of 0.1 N-sodium thiosulphate solution used, subtract 0.00157 from 0.04704 gram of phenol. This determines the quantity of carboic acid in the 25 cc. of distillate taken.

3. Messinger-Vortmann¹ Volumetric Method

Excess of iodine (8 atoms of iodine to 1 molecule of phenol dissolved in 4 molecules of potassium hydroxide), added to a moderately warm alkaline phenol solution, will produce a dark red, non-crystalline precipitate. One molecule of phenol requires 6 atoms of iodine:



This red precipitate dissolves in hot potassium hydroxide solution with a red-brown color and appears as white 2, 4, 6-tri-iodophenol, melting at 154–156°, on addition of an excess of dilute sulphuric acid. Messinger and Vortmann regard the red compound as di-iodophenyl hypoiodite, $\text{C}_6\text{H}_3\text{I}_2\text{OI}$, which potassium hydroxide converts into the more stable isomeric tri-iodophenol.



Procedure.²—The reaction between alkaline phenol solution and iodine is rather slow in the cold but is hastened at 50–60°.

Place a measured volume of aqueous phenol solution (5–10 cc.) in a small flask and add a measured volume of 0.1 N-potassium hydroxide solution until the mixture is strongly alkaline. Warm gently by dipping the flask in water at 60° and add 10–15 cc. more of 0.1 N-iodine solution than the volume of 0.1 N-potassium hydroxide solution used, or until the excess of iodine produces a strong yellow color. Agitation will cause a deep red precipitate to appear. Cool the solution, acidify with dilute sulphuric acid, and dilute to a defi-

¹ J. Messinger and G. Vortmann: A New Class of Iodated Phenols. *Ber. d. Deutsch. chem. Ges.* 22 (1889), 2312, and *Volumetric Estimation of Phenols*. *Ibid.* 23 (1890), 2753. See also Kossler and Penny, *Zeitschr. f. physiol. Chemie* 17 (1892), 117.

² Use 0.5–1 per cent. carboic acid solution for laboratory experiments.

nite volume (250-500 cc.). Filter an aliquot portion (100 cc) rapidly and determine excess of iodine with 0.1 N-sodium thiosulphate solution

Calculation.—Each molecule of phenol requires 6 atoms of iodine. Therefore

$$1 \text{ atom of iodine} = \frac{C_6H_5OH}{6} = \frac{94.05}{6} = 15.675 \text{ grams of phenol}$$
 1000 cc of 0.1 N-iodine solution, containing 0.1 gram-atom of iodine, correspond therefore to 1.5675 grams of phenol.

Note.—This method will not give satisfactory results, unless at least 3 molecules of sodium or potassium hydroxide are taken for 1 molecule of phenol

Estimation of Phenol in Urine¹

In determining carbolic acid in urine, the normal occurrence of phenols must not be overlooked. After a mixed diet, the quantity of normal human urine passed in 24 hours will yield approximately 0.03 gram of phenols (phenol and more especially p-cresol)

In certain diseases where there is excessive bacterial decomposition within the organism, in the intestines for example, urine contains more of these phenols and consequently more conjugated sulphuric acids. Even external application of carbolic acid, for instance the use of carbolic acid water as a lotion, is sufficient to increase the quantity of phenyl-sulphuric acid in urine

Detection of Carbolic Acid in Presence of Aniline

Aniline closely resembles carbolic acid in behavior toward Millon's reagent and bromine water. But the two substances can be easily separated. Add potassium hydroxide solution in large excess and distil. The distillate will contain aniline alone. Or make the solution strongly acid with dilute sulphuric acid and extract with ether which will dissolve only carbolic acid. Evaporate the ether extract at a moderate temperature and examine the residue

Toxic Action and Elimination of Carbolic Acid

Concentrated carbolic acid coagulates and destroys the constituents of the human body, especially proteins and protoplasmic structures. It has therefore a very strong caustic action. But its action is not merely local, for after absorption it shows an affinity particularly for the central nervous system, brain and spinal cord. The first indications of this in animals are strong stimulation, increased irritability as in the case of strychnine, and paralysis. In man the period of stimulation is very slow in appearing. In chronic poisoning, after repeated small doses of carbolic acid, degeneration of the kidneys and liver is a result of absorption. The human organism absorbs carbolic acid very rapidly. Absorption from the skin, the gastro-intestinal tract, abrasions and the respiratory organs takes place readily. In the human organism the poison is converted by conjugation with acid potassium sulphate into potassium phenyl-sulphuric acid, $(C_6H_5)O \cdot SO_3 \cdot OK$. When the quantity of carbolic acid is very large, it is

¹ The methods of estimating volatile phenols of the urine are taken up in Chapter V, page 557 of this book.

also converted into phenyl-glycuronic acid by conjugation with glycuronic acid, $\text{HOOC}(\text{CH}(\text{OH}))_4\text{CHO}$. Considerable carbolic acid is oxidized within the body to dihydroxy-benzenes, namely catechol, $\text{C}_6\text{H}_4(\text{OH})_2(1, 2)$, and quinol, $\text{C}_6\text{H}_4(\text{OH})_2(1, 4)$. These enter into synthesis with sulphuric acid and appear in the urine as ethereal salts of sulphuric acid. The dark color of "carbolic urine" is largely due to further oxidation of quinol, whereby colored products (quinone?) are formed. In carbolic acid poisoning, urine often has a pronounced dark color (greenish to black). Urine in other cases is amber-yellow at first, but standing in air gives it a deeper color. When carbolic acid poisoning is suspected, the urine should be examined chemically. "Carbolic urine"¹ differs from normal human urine in being nearly free from "sulphate-sulphuric acid,"² the so-called "pre-formed sulphuric acid." Consequently barium chloride solution, in presence of excess of acetic acid, gives only a slight precipitate of barium sulphate or none at all. Filter when there is a precipitate and warm the clear filtrate with a few cc of concentrated hydrochloric acid. An abundant precipitate of barium sulphate will usually appear. The mineral acid decomposes phenyl-sulphuric acid into phenol and sulphuric acid which is then precipitated. Normal human urine contains considerably more "sulphate-sulphuric acid" (A-sulphuric acid) than "ethereal sulphuric acid" (B-sulphuric acid). The average proportion between the two being $\text{A-SO}_4 : \text{B-SO}_4 = 10:1$. Barium chloride solution, added to normal urine in presence of acetic acid, produces a heavy precipitate of barium sulphate.

Distribution of Carbolic Acid in Human Body after Fatal Poisoning

Bischoff³ examined organs, removed from a man who had died 15 minutes after taking 15 cc of liquid carbolic acid, and found the poison distributed as stated in the table below. The organs in this case were perfectly fresh. Only a small portion of the stomach was received.

Weight	Organ	Phenol
242 grams	Contents of stomach and intestine	o 171 gram
112 grams	Blood	o 028 gram
1480 grams	Liver	o 637 gram
322 grams	Kidney	o 201 gram
1445 grams	Brain	o 314 gram

Bischoff distilled with steam until the distillate gave no further precipitate with bromine water. The results show how rapidly carbolic acid is absorbed and how soon it is distributed throughout the body.

Urine gives a distinct test for carbolic acid 15 minutes after the poison has been taken by the mouth, or hypodermically. This shows how rapidly carbolic

¹ The methods of examination of "Carbolic Urine" and the estimation of "Sulphate-Sulphuric Acid" and the conjugated sulphuric acid are taken up in Chapter V, page 559 of this book.

² This sulphuric acid, occurring in the urine in the form of sulphates, gives SO_4^{--} -ions.

³ C. Bischoff. Distribution of Poisons in the Human Organism in Cases of Poisoning. Ber. d. Deutsch. chem. Ges. 16 (1883), 1337.

acid is absorbed. Most of the carbolic acid absorbed is eliminated in 4-5 hours. Schaffer¹ found the quantity of conjugated sulphuric acid in urine to increase in exact proportion to the quantity of carbolic acid taken.

Baumann² has published certain facts relating to the quantity of carbolic acid formed during putrefaction of protein substances. Baumann states that he obtained from 100 grams of fresh pancreas and 100 grams of moist fibrin, mixed with 250 cc of water, after 6 days of putrefaction 0.073-0.078 gram of tribromophenol, corresponding to 0.0208-0.022 gram of phenol. Most of the volatile phenols formed during the putrefaction of proteins consists of p-cresol. Brieger³ determined that 0.72 gram of phenol was the maximum quantity formed as the result of 6 days putrefaction of a liver weighing 2 kilograms. If putrefaction was allowed to go on longer, the quantity of phenol decreased.

CRESOLS AND LYSOL

The three cresols, $C_6H_4(CH_3)OH$, together with a small quantity of carbolic acid and hydrocarbons are the chief components of crude coal-tar creosote. Since this mixture is only slightly soluble in water, addition of potash soaps, alkali salts of aromatic acids such as sodium cresotinate, $HO C_6H_3(CH_3)COONa$, renders it soluble. Lysol belongs to preparations of this kind which are frequently used in the treatment of wounds and for purposes of disinfection. As a matter of fact it is identical with the *Liquor Cresoli Saponatus* of the German Pharmacopoeia by which is understood a solution prepared by warming 1 part of crude cresol with 1 part of a potash soap from linseed oil. It is a clear, brown liquid having a cresol-like odor and giving a clear mixture with equal volumes of alcohol, ether and chloroform. When shaken with twice its volume of water, lysol gives a gelatinous mass that dissolves in more water forming a liquid that foams freely. Dilute ferric chloride solution produces a transitory blue-violet color and then a dirty turbidity. Addition of an excess of hydrochloric acid precipitates the cresols and fatty acids as a brown oily liquid.

Detection of Cresols

For the chemical confirmation of suspected poisoning by lysol, it is usually sufficient to detect the presence of the principal component

¹ Journal f prakt Chemie, Neue Folge 18 (1878), 282.

² E. Baumann: Formation of Phenol during the Putrefaction of Proteins. Ber d Deutsch chem. Ges. 10 (1877), 685, Zeitschr. f. physiol. Chem. 1 (1877), 61.

³ L. Brieger: Aromatic Products of the Putrefaction of Albumin. Zeitschr. f. physiol. Chem. 3 (1879), 139.

of lysol, that is, the cresols. This is accomplished by strongly acidifying the material with tartaric or dilute sulphuric acid and distilling with steam. The cresols pass over almost quantitatively. They may be separated from the hydrocarbons that distil over at the same time by extracting the distillate with ether. The residue left upon evaporating the ether solution is shaken with dilute sodium hydroxide solution and petroleum ether. The latter dissolves only the hydrocarbons. The cresols may be precipitated from the alkaline aqueous solution by dilute hydrochloric or sulphuric acid. They are finally extracted with ether and the solution in this solvent is evaporated. A saturated aqueous solution of the ether residue may be used for the following general phenol tests:

1. The colors given by the three cresols with dilute ferric chloride solution are different. The color with o-cresol is blue changing in a few minutes to green, with m-cresol evanescent blue changing to a dirty yellowish brown with turbidity, and with p-cresol a permanent blue-violet.

2. Only o-cresol gives a positive test with Melzer's benzaldehyde test. Consequently by this reaction it is possible to determine whether o-cresol is present in crude coal-tar creosote or in lysol (*Liquor Cresoli Saponatus*) prepared from it.

3. All three cresols give a positive test with Millon's reagent.

4. Lex's hypochlorite test gives first a green and then a blue color with o- and m-cresol; and with p-cresol usually only a dirty greenish color.

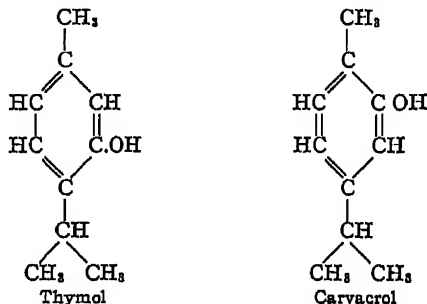
Examination of Urine in Lysol Poisoning

In suspected lysol poisoning, as in carbolic acid poisoning, the urine is first examined chemically and microscopically. According to Fries,¹ lysol poisoning causes severe injury to the kidneys recognizable by excessive elimination of erythrocytes, pigment- and epithelial-casts. Moreover masses of kidney epithelial casts, red and white blood-corpuscles, and much albumin are present. These casts were 3-5 millimeters in length and could be seen with the naked eye as red-brown structures on the bottom of the urine-glass. In lysol poisoning both conjugated aromatic sulphuric and glycuronic acids of the urine are greatly increased. Consequently the normal ratio between conjugated sulphuric acid and sulphate-sulphuric acid is altered in favor of the former.

¹ Fries: Münchener med. Wochenschr. 1904, 709

THYMOL

The two isomeric methyl-isopropyl-phenols, thymol and carvacrol, $C_6H_3(CH_3)(C_3H_7)OH$, differ from each other, as may be seen from their structural formulae:



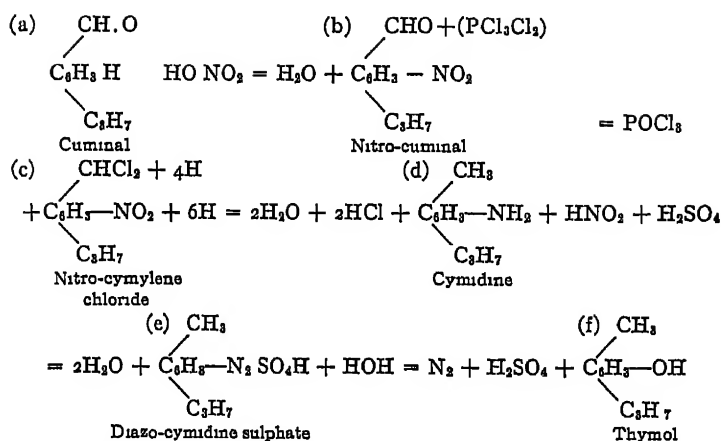
Thymol is 1,4,3- and carvacrol 1,4,2-methyl-isopropyl-phenol.

Thymol is a component of different essential oils. Together with cymene, $C_{10}H_{14}$, and thymene, $C_{10}H_{16}$, it occurs to the extent of 20-50 per cent. in essential oil of *Thymus vulgaris*; 50-60 per cent. in the North American labiate, *Monarda punctata*, about 40 per cent. in the Japanese labiate, *Mosula japonica*; and 50-60 per cent. in the oil from the fruit of the East Indian umbellifera, *Ptychotis ajowan*. Together with a small quantity of carvacrol, thymol also occurs in the oil of *Thymus Serpyllum*.

Isolation.—Shake the given essential oil with warm 30 per cent. sodium hydroxide solution (sp gr 1.330) and after several hours dilute the mixture with three times its volume of hot water. Separate the precipitated hydrocarbons from the aqueous solution of sodium thymolate, $C_6H_3(CH_3)(C_3H_7)ONa$, and decompose the latter with hydrochloric acid. Draw off the thymol which separates as an oil, free it from water, distil and set the distillate in a cool place to crystallize. Thymol may also be recrystallized from glacial acetic acid.

Synthesis of Thymol.—This may be brought about by starting with cuminal.¹ Cuminal, or isopropyl-benzaldehyde, $(CH_3)_2CH.C_6H_4.CHO$, is purified by means of its sodium bisulphite compound. The product thus obtained is then converted into nitro-cuminal by treating it with a mixture of fuming nitric acid and concentrated sulphuric acid with cooling. The aldehyde oxygen in this compound is replaced by chlorine through the agency of phosphorus pentachloride and the resulting nitro-cymylene chloride is then reduced to cymidine by means of zinc and hydrochloric acid. In the form of its sulphate the latter is diazotized in the usual manner in sulphuric acid solution with sodium nitrite. Diazotized cymidine by boiling with water is converted into thymol which is finally obtained pure by distillation with steam:

¹ O. Widmann: Synthesis of Thymol from Cuminal. Ber. d. Deutsch. chem. Ges. 15 (1888), 166.



Properties.—Thymol forms large, colorless, hexagonal crystals that melt at 50–51°, boil at 230° and have the odor of thyme. Thymol crystals (sp. gr 1.028) sink in water, whereas melted thymol floats on water. Even at 100° thymol vaporizes rather rapidly and consequently distills easily with steam. When fused or distilled, thymol frequently remains liquid for some time. Thymol dissolves in about 1200 parts of water and in less than 1 part of alcohol, ether and chloroform. It is readily soluble in solutions of caustic alkalis.

Use and Physiological Behavior.—Thymol acts as a preventive of fermentation and putrefaction but its strength as an antiseptic is less than that of carbolic acid and salicylic acid. It is an excellent intestinal disinfectant but the fatty acid esters of thymol, such as thymol palmitate, $\text{C}_{16}\text{H}_{31}\text{COOC}_6\text{H}_4(\text{C}_6\text{H}_7)(\text{CH}_3)$, are more active and at the same time less toxic than thymol itself. For external use thymol serves as a substitute for carbolic acid in the treatment of wounds and also in chronic skin diseases. It is a component of many lotions used for the mouth and teeth. As a rule the usual medicinal doses of thymol, aside from local irritations, produce no dangerous secondary effects. In the case of certain individuals ringing in the ears, headache and violent delirium have been observed. Even collapse accompanied by paralysis and somnolence appeared. Larger doses of thymol have a paralyzing action upon animals. The post-mortem appearances in such cases, as a result of the action of thymol, are fatty liver, and irritation of the kidneys and lungs (Kobert). Like phenol, after previous conjugation, thymol is eliminated in the urine as thymol-sulphuric acid, thymol-glycuronic acid, and after oxidation partly as thymo-quinol-sulphuric acid. In thymol-urine there is the further occurrence of the chromogen of a green pigment that becomes indigo-blue when heated with hydrochloric acid and purple-red on the subsequent addition of ammonia. This blue dyestuff can be extracted with chloroform. (Blum.¹) The phenol-glycuronic acid of thymol-urine can be best detected in the form of the water insoluble dichloro-thymol-glycuronic acid, $\text{C}_6\text{HCl}_2(\text{CH}_3)(\text{C}_6\text{H}_7)\text{OCH}(\text{OH})(\text{CH}_2\text{OH})_4\text{COOH}$ (?). To produce this compound, concentrated hydrochloric acid and sodium hypochlorite solution are added

¹ F. Blum: Thymol-Glycuronic Acid. *Zeitschr. f. physiolog. Chemie* 16 (1892), 514.

to the usually green colored thymol-urine until the green color has disappeared. After rather long standing, within 4 days, long crystalline needles separate and are dissolved in sodium carbonate solution. After extracting this solution with ether, the conjugated dichloro-glycuronic acid may be precipitated by dilute sulphuric acid in fine white needles. This compound is a monobasic acid having the formula, $C_{16}H_{22}Cl_2O_8$, and melting at $125-126^\circ$. It is insoluble in cold water but easily soluble in alcohol, ether, acetone and benzene. As a phenol it is also readily soluble in solutions of caustic alkalies. This acid is laevo-rotatory; $\alpha_D-66^\circ 11'$ for an alcoholic solution. It reduces neither Fehling's nor ammoniacal silver nitrate solution. When boiled with 5 per cent sulphuric acid, it undergoes cleavage into glycuronic acid and 1, 6-dichloro-thymol. In the latter compound the two chlorine atoms are in the ortho-position to the methyl group.

Detection of Thymol

To detect thymol with greater certainty, acidify the material first with tartaric or dilute sulphuric acid and then distil. Thymol passes over rather easily with steam and usually separates in the distillate in the form of oil-drops, since it dissolves in water only with very great difficulty (1.1200). Then extract the distillate, which may have the thyme-like odor of thymol, with ether, evaporate or distil the ether extract, and test the residue for thymol by the following reactions:

1. Concentrated sulphuric acid dissolves thymol, giving in the cold a colorless or faintly yellowish solution that takes on a beautiful rose-red color when gently warmed.

2. Dissolve a small crystal of thymol in 1 cc. of glacial acetic acid and carefully pour this solution upon 6 drops of concentrated sulphuric acid and 1 drop of officinal nitric acid (25 per cent.). The last two acids are at the bottom of the test-tube and a blue-green zone appears at the contact-surface of the two liquids. If the contents of the tube are shaken around, the mixture appears blue-green by reflected light and red-violet by transmitted light. The mixture is fluorescent.

3. Piria's Test.—Neither the aqueous nor the alcoholic solution of thymol gives a color with ferric chloride. But if a solution of thymol in concentrated sulphuric acid (see above) is poured into 10 volumes of water and the mixture is allowed to stand with frequent shaking at $30-35^\circ$ with an excess of white lead, the neutral filtrate gives a beautiful violet color with a little ferric chloride solution.

4. Eykmann's Test.—If a few drops of Eykmann's reagent are added as an upper layer to a solution of thymol in concentrated sulphuric acid, a red zone appears. Then if the contents of the tube

are well-cooled and mixed, the mixture first turns red, then immediately green. Upon dilution with water the red color reappears, changing to yellow upon subsequent addition of ammonia

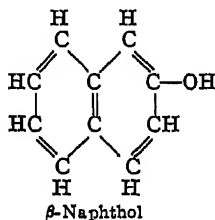
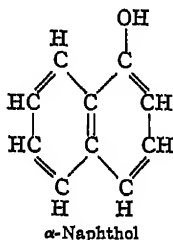
5. Liebermann's Test.—Liebermann's reagent colors a solution of thymol in concentrated sulphuric acid first red and then almost immediately green

6. Lustgarten's Test.—If a solution of thymol in a little 50 per cent potassium hydroxide solution is only gently warmed and a few drops of chloroform are added, the mixture will have a beautiful violet-red color.

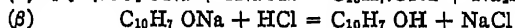
In an aqueous solution of thymol, bromine water produces only a turbidity but gives no crystalline precipitate (distinction from carboic acid). An aqueous thymol solution does not give with Millon's reagent the red color so characteristic of monacid phenols.

NAPHTHOLS

As in the case of all mono-substitution products of naphthalene, there are also two isomeric monoxy-naphthalenes, $C_{10}H_7OH$, α - and β -naphthol



Preparation.—Both naphthols are prepared by fusing the sodium salts of the corresponding naphthalene sulphonic acids with sodium hydroxide, dissolving the melts in water, adding hydrochloric acid in excess to the solutions, and recrystallizing the precipitated naphthols from boiling water, after first washing with water, or purifying by sublimation



α -Naphthol.—This compound crystallizes in colorless, shining, silky needles that melt at 95° and have a characteristic odor. It sublimes with gentle heat and is also volatile with steam. It is only slightly soluble in water but readily soluble in alcohol, ether and chloroform.

β -Naphthol.—This compound crystallizes in white, shining, rhombic leaflets melting at 122° . It easily sublimes when more strongly heated and is also volatile with steam. It dissolves in 1000 parts of cold and in 75 parts of hot water, and is readily soluble in alcohol, ether and chloroform. Solutions of β -naphthol in sodium or potassium hydroxide and ammonia exhibit blue fluorescence

Medicinal Use and Toxic Action of β -Naphthol

In the form of a 10-15 per cent. ointment, β -naphthol is used principally in skin diseases. Naturally in rubbing it over the entire body abundant absorption takes place. Lewin, who had a patient with itch rubbed with 15 grams of β -naphthol, observed the appearance of eczema followed after 14 days by acute nephritis with albuminuria. On the other hand, an 8 year old and a 6 year old boy were treated for scabies with 2 per cent β -naphthol ointment, the elder receiving in all 4 grams and the younger 3 grams of the ointment. After 20 days both became ill from acute nephritis accompanied by oedema. The younger died and the post-mortem confirmed the diagnosis. Consequently in addition to severe irritation of the skin and mucous surfaces, β -naphthol after absorption may also give rise to acute effects in the blood, central nervous system, and especially in the kidneys (Kobert).

Behavior of β -Naphthol and Naphthalene in Organism.

According to Edlefsen,¹ naphthalene in doses of 0.5-0.75 gram is oxidized in the organism to β -naphthol which is then eliminated in the urine mostly as conjugated glycuronic acid and in smaller quantity as ethereal sulphuric acid. After administration of small doses of β -naphthol, 0.3-0.5 gram, β -naphthol-glycuronic acid is only exceptionally found in the urine, since most of the β -naphthol that gets into the circulation through absorption is eliminated as ethereal sulphuric acid. Benzo-naphthol, or β -naphthol-benzoate, $C_{10}H_7 \cdot COOC_6H_5$, administered in small and medium doses of 0.6, 0.9 or 1.2 grams, is almost always eliminated as ethereal sulphuric acid. To bring about the hydrolytic cleavage of the latter and detect β -naphthol, the given urine is boiled for some time under a reflux with 10 cc. of concentrated hydrochloric acid. The β -naphthol is then extracted with ether and the ether solution distilled or evaporated. The residue that remains can then be tested for β -naphthol by the reactions given below. To test urine direct for β -naphthol, add according to Edlefsen to 5-10 cc. of urine 3-6 drops each of calcium hypochlorite solution and hydrochloric acid. Owing to the formation of naphthoquinone the color of the urine becomes lemon-yellow. Extract this dyestuff with ether and add some of the ether extract to 1 per cent aqueous resorcinol solution. A red ring will appear. A β -naphthol-urine also shows blue fluorescence upon addition of sodium or potassium hydroxide solution or ammonia.

Detection of α - and β -Naphthol

1. Calcium hypochlorite solution gives a violet color with an aqueous α -naphthol solution, but is without effect upon a solution of β -naphthol.
2. Chlorine water produces a white precipitate with an aqueous α -naphthol solution. This precipitate dissolves in ammonia with a bluish color. Under the same conditions β -naphthol produces a white turbidity that disappears with an evanescent green color upon addition of ammonia, the solution becoming colorless.

¹ G. Edlefsen: Experiments upon Elimination and Detection of β -Naphthol in Urine after Administration of Small Doses of Naphthalene, Benzo-Naphthol and β -Naphthol. *Archiv f. experim. Pathol u. Pharmacol.* 82 (1905), 429.

3. Ferric chloride solution added to an aqueous solution of α -naphthol gives a white precipitate of α -dinaphthol, $C_{20}H_{18}(OH)_2$, that soon turns violet, whereas aqueous β -naphthol solution takes on a greenish color and gradually white flocks of β -dinaphthol separate

4. Pira's Test.—Warm α -naphthol at $80-100^\circ$ for some time upon the water-bath with four times the quantity of concentrated sulphuric acid. Dilute this solution with about 10 times the quantity of water and neutralize with white lead. Ferric chloride solution will impart a beautiful green color to the filtrate, whereas β -naphthol gives a violet or beautiful blue color.

5. Chloral Hydrate Test.—Warm α -naphthol with 20 times the quantity of chloral hydrate upon the water-bath for 10 minutes. The mixture has a ruby-red color and the product of the reaction is soluble in alcohol with a red color. Under the same conditions β -naphthol gives a mixture having a deep blue color and soluble in alcohol with blue color.

6. Formaldehyde Test.—Dissolve 0.1 gram of α -naphthol in a little sodium hydroxide solution and warm after adding a few drops of formaldehyde solution. The color of the mixture is green and soon changes to blue. This test is given neither by β -naphthol nor by a mixture of the two isomeric naphthols.

7. Lustgarten's Test.—The solution of α -naphthol in a little concentrated potassium hydroxide solution gives a blue-violet color when warmed with a few drops of chloroform. A pure blue color is given by β -naphthol.

8. Eykmann's Test.—A black-green ring is given by α -naphthol, whereas a beautiful green fluorescing color-zone appears with β -naphthol.

9. Millon's Test.—Even in the cold, α -naphthol gives a beautiful scarlet-red color, whereas β -naphthol produces more of a reddish brown color with separation of a flocculent precipitate.

10. Diazo-Reaction.— α -Naphthol gives an intense purple-red and β -naphthol a beautiful scarlet-red color.

11. Jorissen's Test.—Addition of about 5 cc. of 0.1 N iodine solution and an excess of sodium hydroxide solution to 0.1 gram of α -naphthol produces a turbid, intense violet solution, but β -naphthol gives a clear colorless solution.

12. Reverse Molisch's Test.—Add as an upper layer to concentrated sulphuric acid a few drops of an alcoholic solution of α -naphthol containing very dilute sugar solution. A violet ring will appear. If the entire mixture is shaken while cooling, it will become violet- to purple-red. This test is not given by β -naphthol.

Isolation of the Two Naphthols

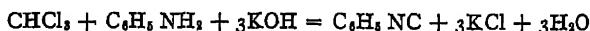
To separate either or both of these two naphthols from material, for example, from urine, acidify it with dilute sulphuric acid and distil. Extract the distillate with ether, evaporate or distil off the ether, and examine the residue for α - or β -naphthol. If the residue is not too small, it may be purified by dissolving in alcohol, heating the solution with bone-black, and evaporating the filtrate.

CHLOROFORM

Chloroform distils easily with steam and appears in the first fraction in largest quantity. When much chloroform is present, it will

separate from the distillate as heavy, colorless globules, whereas a small quantity will remain in solution. This solution usually has the characteristic odor and sweetish taste of chloroform. In a fatal case of suspected chloroform poisoning, the detection of this compound, especially in blood, brain, and also in urine, should be undertaken as soon as possible, since chloroform on account of its great volatility may completely and easily disappear from the body. Chloroform may be detected in the distillate by the following tests

1. Hofmann's Phenyl-isocyanide Test.—Add 1–2 drops of aniline to the chloroform solution and then a few cc. of aqueous or alcoholic potassium hydroxide solution. Gentle heat will produce phenyl-isocyanide (C_6H_5NC). The penetrating and repulsive odor of this compound is easily recognized. This is a very delicate test by which 1 part of chloroform dissolved in 6000 parts of alcohol may be detected with certainty (A. W. Hofmann):



Notes.—Chloral, chloral hydrate, bromal, bromoform, iodoform and tetrachloro-methane also give this test

The fact that aniline boiled with potassium hydroxide solution gives a peculiar faintly ammoniacal odor, even when chloroform is absent, must not be overlooked. There is small chance, however, of confusing this odor with the repulsive smell of phenyl-isocyanide. In doubtful cases warm some water, containing a drop of aniline and a trace of chloroform, with potassium hydroxide solution and compare the odor with that in question

2. Schwarz's¹ Resorcinol Test.—Dissolve about 0.1 gram of resorcinol in 2 cc. of water, add a few drops of sodium hydroxide solution, and finally the liquid containing chloroform. This mixture heated to boiling will develop even in very dilute solution a yellowish red color attended by a beautiful yellowish green fluorescence.

Chloral, bromal, bromoform and iodoform also give this test

3. Lustgarten's² Naphthol Test.—Dissolve a few centigrams of α - or β -naphthol in 1–2 cc. of 33 per cent aqueous potassium hydroxide solution. Warm to 50° and add the solution to be tested. If chloroform is present, the mixture will become blue or blue-green. With β -naphthol the color is less stable, changing in the air first to green and then to brown. Acidification of the blue liquid produces

¹ Schwarz *Fresenius Zeitschr. f. analyt. Chem.* 27, 668

² S. Lustgarten *Detection of Chloroform, Iodoform and Naphthol in Animal Liquids and Organs* *Monatsh. f. Chem.* 3 (1882), 715.

a brick-red precipitate, consisting of a mixture of naphthol and a red dyestuff.

Chloral, chloral hydrate, bromal, bromoform and iodoform also give this test

4. Fujiwara's¹ Pyridine Test.—Mix 2 cc of pyridine with 3 cc. of 10 per cent. sodium hydroxide solution, heat to boiling and add 1 cc of the liquid to be tested. Even a trace of chloroform will produce a bright, blue-red color.

Chloral, bromoform, iodoform and several similar compounds also respond to this test

One part of chloroform in 1,000,000 parts of water, 500,000 parts of ether, or 300,000 parts of alcohol can be detected by this test. It is equally sensitive toward the other substances mentioned.

5. Reduction Tests.—(a) **With Fehling's Solution.**—Warm the liquid containing chloroform with Fehling's solution. A red precipitate of cuprous oxide will appear.

(b) **With Ammoniacal Silver Nitrate Solution.**—Add excess of ammonium hydroxide to silver nitrate solution and then the liquid containing chloroform. Heat will produce a black precipitate of metallic silver.

These reactions are not characteristic of chloroform, because many volatile organic substances, such as formic acid and aldehydes, that may occur in distillates from animal material, reduce Fehling's and ammoniacal silver nitrate solution.

6. Cyanide Test.—This test is based upon the conversion of chloroform into ammonium cyanide when heated with alcoholic ammonia:



Seal the liquid to be tested for chloroform in a glass tube (pressure-tube²) with a little solid ammonium chloride and alcoholic potassium hydroxide solution. Not more than a quarter of the tube should be filled with liquid. Heat for several hours in a boiling water-bath. Cool the tube, remove the solution and test for hydrocyanic acid by the Prussian blue reaction. A positive test means that the distillate contained chloroform, provided that preformed hydrocyanic acid was not present in the material.

¹ Chemical Abstracts 11 (1917), 3201. Sitzungsber. u. Abhandl. Naturforsch. Ges. Rostock, 6 (1914), 1. See also J. H. Ross, J. Biol. Chem. 58 (1923-24), 641.

² An ordinary citrate of magnesium bottle is a convenient apparatus for this test. Wrap a towel around the bottle, place it in the water-bath, and gradually raise the temperature to boiling. Do not remove the bottle until it is cold. Tr.

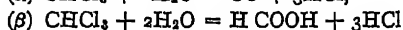
Estimation of Chloroform in Parts of Cadavers

Mix a weighed portion of material with water containing tartaric acid and distil as long as there is any chloroform. To determine when this point is reached, apply the phenyl-isocyanide test to a few cc. of liquid collected at the end of distillation. Add some calcium carbonate to combine with free hydrochloric acid. Warm the distillate to about 60° and draw washed air through it by suction. Pass the air through a combustion-tube heated at a high temperature and then into silver nitrate solution acidified with nitric acid. Weigh the precipitated AgCl (N).

Calculation.



This method is based upon the fact that chloroform heated with steam above 200° is decomposed into carbon monoxide, hydrochloric and formic acid:



In a series of blank experiments B Fischer¹ has shown that the stomach, stomach-contents and blood of a person, who has not taken chloroform, give no volatile chlorine compounds under these conditions. By this method B Fischer found in the cadaver of a laborer, who had died during chloroform narcosis, the following quantities of chloroform

Weight	Organ	Chloroform
985 grams	Stomach and contents and parts of the intestine	0.1 gram
780 grams	Lungs and blood from the heart	0.055 gram
445 grams	Portions of spleen, kidneys and liver	traces
480 grams	Brain	0.07 gram

From these results it appears that most of the chloroform was in the brain and blood.

Quantitative Estimation of Chloroform by Fujiwara's Test

Cole² has recently applied the pyridine test for chloroform, described by Fujiwara and Ross,³ to the quantitative estimation of small amounts of chloroform either in aqueous solutions, or in acidified (0.1 per cent HCl) extracts of animal tissues

¹ B Fischer Jahresber. d. chem. Untersuchungsamtes d. Stadt Breslau f. d. Zeit from April 1, 1894, until March 31, 1895.

² W. H. Cole. The Pyridine Test as a Quantitative Method for the Estimation of Minute Amounts of Chloroform. Jour. of Biolog. Chem. 71 (1926), 173

³ J. H. Ross J. Biol. Chem. 58 (1923-24), 641

Procedure.—Measure from a burette into a narrow test-tube (10 cc capacity) 2 cc. of 20 per cent sodium hydroxide solution. Add also from a burette 1 cc. of chemically pure pyridine (colorless) and from a 1 cc pipette 1 cc. of the solution to be tested. Loosely stopper the test-tube to prevent evaporation of pyridine, shake constantly, and immerse for 1 minute in water at 100° well above the liquid level. Then immerse in cold water and reduce the temperature to 20°. The pyridine and test solution, colored pink or red if chloroform is present, rise above the alkali during cooling. Transfer the former by means of a 1 cc pipette to a color comparison tube and compare the solution in a comparator with color standards previously made up to match the colors obtained from known concentrations of chloroform.

In quantitative work it is essential that the amounts of pyridine and test-solution be measured accurately, since the color produced is a function of the volume over which it is distributed. Since pyridine is volatile (Bpt 115°), long, loosely stoppered tubes are necessary to keep the volume of pyridine as constant as possible. The color comparison tubes must be of the same diameter and of the same kind of glass as that used for the color standards.

Cole has found that chloroform in concentrations from 0.1 per cent. (by volume at 15°) down to 0.0001 per cent. (1 part of chloroform in one million parts of water) may easily be detected. In his original paper the author gives further details with regard to the preparation of color standards, as well as certain precautions that should be observed in carrying on quantitative estimations by this method.

Behavior of Chloroform in Human Organism

During chloroform narcosis, this anaesthetic when inhaled first passes from the air into the blood-plasma which then transmits it to the red blood-corpuscles where it may accumulate in relatively large quantity. Air passed through blood will remove chloroform completely. Pohl (see Kobert's "Intoxikationen") states that blood may contain 0.62 per cent of chloroform, three-fourths of which will be in the red blood-corpuscles. At the height of a harmless narcosis the blood contained only 0.035 per cent of chloroform. Absorption of chloroform takes place from all parts of the body. The stimulative action of chloroform upon the mucous membranes of the respiratory passages explains such disturbances as coughing, secretion of saliva and reflex slowing of respiration and heart-beat, occurring at the beginning of narcosis. Dilatation of the blood-vessels of organs living after death is due to paralysis caused by even small doses of chloroform. A drop in blood-pressure accompanies paralysis of the brain and the heart's action is feeble and slower. Several researches with regard to the effect of inhaled chloroform upon human and animal metabolism have shown an increase in the quantity of nitrogen in the urine after prolonged narcosis because more protein is decomposed. The amount of neutral sulphur and chlorine in the urine also increases. The increase of the latter is due in part at least to the conversion of chloroform into chloride. The acidity of the urine is also much higher. A final characteristic of chloroform urine is the high content of reducing substances. The increased protein decomposition in chloroform narcosis affects both reserve protein and that of the tissues. This may explain degeneration in red blood-corpuscles, glandular organs, the heart, etc., after frequent narcoses or one of long duration.

The temporary or permanent paralysis of isolated animal or vegetable cells, such as leucocytes, ciliated cells, yeast cells, algae and spores, is evidence of the antiseptic action of chloroform when present in proper concentration in air or in a liquid. This explains the use of 1 per cent aqueous chloroform solution as an antiseptic. Added to urine it acts as a preservative. Therefore it may be used in the study of the action of enzymes but not of bacteria, though all micro-organisms are not paralyzed or killed by chloroform water.

Distribution of Chloroform in Cadaver

A large part of the absorbed chloroform undergoes decomposition in the body and a part is eliminated by the lungs. Pohl and Hans Meyer have studied the distribution of chloroform in the body and have found that the red blood-corpuscles and the brain are most likely to show this poison. After chloroform has been inhaled, some will appear in the gastric juice but at most only traces in the urine. In but two out of 15 cases of chloroform narcosis was this poison found in the urine and then only in traces.

Robert states that as a rule it is the exception to find chloroform itself in the cadaver, because part of the poison is converted into chloride in the human organism and part is quickly exhaled during respiration. Usually it is possible to detect chloroform in the breath of patients even 24 hours after narcosis. Büdinger states that the mucus of the respiratory passages retains chloroform.

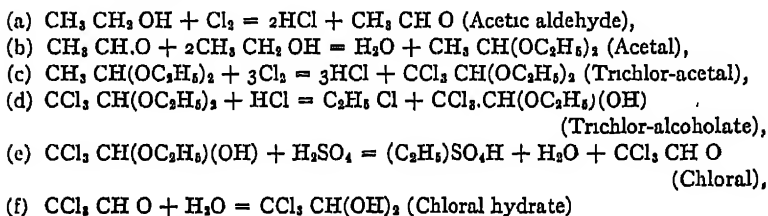
CHLORAL HYDRATE

Chloral hydrate, $\text{CCl}_3\text{CH}(\text{OH})_2$, forms transparent crystals that are dry, permanent and colorless. It dissolves with ease in water, alcohol and ether, and less easily in chloroform, carbon disulphide and fatty oils. Chloral hydrate has a pungent odor and a faintly bitter taste. From an aqueous solution acidified with tartaric acid it distills very slowly with steam. Therefore the complete distillation of a large quantity of chloral hydrate requires considerable time. Chloral hydrate appears as such in the distillate. If the reaction of the material is alkaline during distillation, the distillate will not contain chloral hydrate but chloroform as a resulting product of its decomposition.

Preparation.—Pass dry chlorine into 96 per cent alcohol until the evolution of hydrogen chloride diminishes. At the outset the alcohol should be cooled but toward the end of the process a temperature of about 60–70° is advisable. When the action of chlorine upon the alcohol is complete, the reaction-product will be a crystalline mass when cold. In the main it will consist of chloral-alcoholate and some chloral hydrate (see below). To prepare pure chloral, warm this crude product with about the same volume of concentrated sulphuric acid in a flask under a reflux as long as hydrogen chloride is given off. The chloral that separates is withdrawn and purified by distillation. It boils at 96–97°. Chloral hydrate is formed with considerable evolution of heat by mixing 100 parts

of chloral with 12 parts of water. If this mixture of chloral and water warmed to about 50° is mixed with about half its volume of warm chloroform, chloral hydrate upon cooling will separate in loose crystals. These recrystallized from warm chloroform, carbon disulphide or petroleum benzine will give well-formed crystals.

Explanation.—In the first phase of the reaction chlorine converts alcohol into acetic aldehyde. The latter then in presence of more alcohol (2 molecules) may condense forming acetal. Chlorine converts acetal into trichlor-acetal, the final substitution-product, from which under the influence of hydrogen chloride trichlor-alcoholate and ethyl chloride are produced. Then trichlor-alcoholate warmed with concentrated sulphuric acid yields chloral which finally combines with water forming chloral hydrate.



The action of chlorine upon alcohol is expressed, though not entirely, by equations a-d, for unquestionably secondary reactions take place accounting for the formation of ethylidene chloride, CH_3CHCl_2 , methyl-chloroform, $\text{CCl}_3\text{-CH}_3$, trichlor-acetic acid, CCl_3COOH , and other chlorinated products

Behavior of Chloral Hydrate in the Animal Body

Because of the ease with which chloral hydrate undergoes decomposition, tests for its presence in the cadaver should be made as soon as possible after death. for after putrefactive processes have set in the chances of finding it are not very promising. Stomach-contents and vomitus are especially suitable material for its detection. Chloral hydrate probably undergoes change in the liver. As a result after 2 hours, but sometimes not until 18 hours have elapsed, the urine¹ contains urochloralic acid, as well as a trace of unaltered chloral hydrate, the latter probably appearing only after very large doses. Consequently blood and urine seldom contain more than traces of chloral hydrate. Detection of urochloralic acid in the urine therefore is probably the most reliable means of reaching a decision with regard to suspected chloral poisoning.

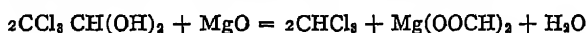
Detection of Chloral Hydrate

Chloral hydrate like chloroform will give the phenyl-isocyanide, resorcinol, naphthol and pyridine tests. But the distillate containing chloral hydrate does not have the characteristic chloroform odor which is also scarcely perceptible in very dilute aqueous chloroform

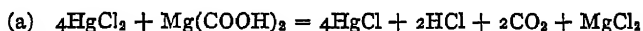
¹The method of detecting urochloralic acid in chloral urine is taken up in Chapter V, page 555, of this book

solutions. In distinction from chloroform, chloral hydrate gives the aldehyde-reaction with Nessler's reagent. Add a few drops of this reagent to an aqueous chloral hydrate solution and shake. It will produce a yellowish red precipitate, the color of which will change after a while to a dirty yellowish green.

When the quantity of chloral hydrate is not too small, it may also be detected by the following procedure. Heat a portion of the distillate for 30 minutes under a reflux with calcined magnesium oxide upon a boiling water-bath. Magnesium formate and chloroform are produced by decomposition of chloral hydrate.

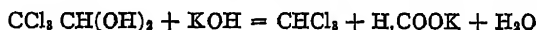


Both products of this reaction, chloroform and formic acid, may be detected by distilling off a few cc. of the solution and testing for chloroform by the phenyl-isocyanide, resorcinol, naphthol and pyridine reactions. Filter the residue from distillation, concentrate the filtrate to a few cc by evaporation and divide into two portions to test for formic acid. Warm one portion with a few drops of mercuric chloride solution which will give a white precipitate of mercurous chloride, if formic acid is present, and heat the other with a little silver nitrate solution which will give a black precipitate of metallic silver if the filtrate contained formic acid. The following equations explain these two tests:



Quantitative Estimation of Chloral Hydrate

(a) The following method gives satisfactory results, if pure aqueous chloral hydrate solutions are used and the excess of potassium hydroxide solution taken is titrated at once. Under these conditions chloral hydrate is decomposed in the following manner:

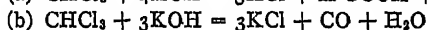


Procedure.—Shake the aqueous chloral hydrate solution well for a few minutes with an excess of n-potassium hydroxide solution, add phenol-phthalein as indicator, and at once titrate the excess of alkali with n-acid.

Calculation.—According to the above equation, 1000 cc. of n-alkali correspond to 1 gram-molecule = 165.38 grams of chloral hydrate.

(b) In dealing with a distillate from any material of known amount, heat it under a reflux for 5–6 hours with an excess of chlorine-

free, alcoholic potassium hydroxide and then evaporate upon the water-bath. Dissolve the residue in water, acidify with dilute nitric acid, precipitate chlorine with silver nitrate, and weigh as silver chloride. Under these conditions alcoholic potassium hydroxide solution converts all chlorine, first separated as chloroform, into potassium chloride:



Consequently 3 molecules of silver chloride (430.14) correspond to 1 molecule of chloral hydrate (165.38). Obviously this method of estimation is applicable only when the distillate, in which chloral hydrate is to be determined, contains neither hydrochloric nor hydrocyanic acid.

Quantitative Estimation of Chloral Hydrate in Blood and Tissues

(Archangelsky¹)

Distil the material for 12–20 hours with its own weight of 20 per cent phosphoric acid, repeating the process if the distillate is turbid or yellow. To complete the decomposition of chloral hydrate into chloroform and formic acid, add 50 cc. of sodium hydroxide solution to the distillate and concentrate upon the water-bath to about 20 cc. Neutralize the solution exactly and heat for 6 hours upon the water-bath with an excess of mercuric chloride solution. Finally weigh the precipitated mercurous chloride. Satisfactory results were obtained by this method when known quantities of chloral hydrate were added to blood and organs. Using this method, Archangelsky has shown that chloral hydrate is not uniformly distributed in the blood but is contained especially in the blood-corpuscles. When narcosis begins, there is less chloral hydrate in the brain than in the blood. But later the percentage of the poison in the brain is higher than in the blood. Archangelsky has further shown how much chloral hydrate the blood must contain before narcosis can appear. A dog's blood must contain 0.03–0.05 per cent. When the blood contains 0.12 per cent, respiration ceases.

Butyl-Chloral Hydrate.—This compound which has the formula, $\text{CH}_3\text{CHCl}\cdot\text{CCl}_2\text{CH(OH)}_2$, has a limited use in medicine as a hypnotic and anti-neuralgic. It melts at 78° and forms white leaflets having an aromatic odor and bitter taste. It is rather difficultly soluble in cold water but dissolves readily in alcohol and ether. Since it is easily volatile with steam, it passes into the distillate when material is faintly acidified and distilled. With caustic alkalis it yields no chloroform and so does not give the phenyl-isocyanide test. This behavior serves to differentiate it from chloral hydrate which it otherwise resembles. The products formed as a result of decomposition by alkalis are dichloro-propylene, hydrochloric acid and formic acid. On the other hand, it reacts with Nessler's reagent like chloral hydrate, forming a red-brown precipitate.

¹ C. Archangelsky. Distribution of Chloral Hydrate and Acetone in the Organism. Arch. f. exper. Path. u. Pharm. 46 (1901), 347.

Butyl-chloral hydrate appears in urine as urobutyl-chloral acid which can be isolated from urine by the method given for urochloral acid. Urobutyl-chloral acid crystallizes in shining, silky, star-shaped needles, is easily soluble in water, alcohol and ether, turns the plane of polarized light to the left, and reduces Fehling's solution after it has first been boiled with dilute acids. When boiled with dilute mineral acids, urobutyl-chloral acid takes up water, undergoing cleavage into trichloro-butylalcohol and glycuronic acid



Therefore like urochloral acid it belongs to the conjugated glycuronic acids. From a physiological point of view, urochloral and urobutyl-chloral acid are of especial interest, since the chlorinated constituents of both, trichloro-ethyl alcohol and trichloro-butyl alcohol, arise in the organism from their corresponding aldehydes as a result of a process of reduction, and not until this has taken place can conjugation with glycuronic acid occur.

iodoform

Iodoform, CHI_3 , crystallizes in shining, hexagonal leaflets or plates. It may also appear as a rather fine crystalline powder, lemon-yellow in color and having a penetrating odor somewhat like saffron. The melting-point of iodoform is approximately 120° . It is nearly insoluble in water, soluble in 70 parts of cold and in about 10 parts of boiling alcohol, and soluble in 10 parts of ether. It is also freely soluble in chloroform. When iodoform is heated violet, vapors of iodine appear.

Detection of Iodoform

Iodoform distils quite easily with steam and gives a milky distillate having a characteristic odor. Extract this distillate with ether and carefully test the residue left after spontaneous evaporation of the solvent. If much iodoform is present, it will form yellow hexagonal plates. To identify iodoform, dissolve this residue, in case it has the odor of this substance, in a little warm absolute alcohol and apply Lustgarten's¹ test:

Lustgarten's Test.—In a rather small, narrow test-tube heat gently over a small flame very little potassium phenolate, that is, a solution of crystallized phenol in concentrated potassium hydroxide solution, with 2–3 drops of the alcoholic solution of the residue. If iodoform is present, a red deposit due to formation of rosolic acid will appear on the bottom of the tube. A few drops of dilute alcohol will dissolve this precipitate with a carmine-red color.

¹ S Lustgarten. Detection of Chloroform, Iodoform and Naphthol in Animal Fluids and Organs *Monatsh f. Chem.* 3 (1882), 715

The phenyl-isocyanide, resorcinol and pyridine tests should also be made as described under chloroform (see pages 60 and 61).

Behavior in the Organism.—Too large doses of iodoform, administered externally as well as internally, have repeatedly given rise to fatal poisonings. Stomach-contents, kidneys, brain, blood and urine should especially be examined if poisoning from iodoform is suspected. In one case death ensued within 7 days following the internal administration of 5 grams of iodoform. The decomposition of iodoform gradually takes place in the organism and iodine is slowly eliminated in the urine and saliva and in small quantity also in the faeces. A noteworthy fact observed by Lustgarten is that iodoform cannot be detected either in the urine or blood of dogs poisoned by this compound.

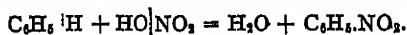
Detection of Iodine in Urine in Iodoform Poisoning

In a suspected case of iodoform poisoning, when this substance can no longer be detected as such, the urine should be examined for iodine. Render about 100 cc of urine strongly alkaline with sodium carbonate, add 1-2 grams of potassium nitrate, evaporate and heat the residue until it has fused and all particles of carbon have disappeared. On account of the volatility of alkaline iodides, carefully avoid heating the residue too strongly and for too long a time. Add chloroform to an aqueous solution of the cold melt, acidify while cooling with dilute sulphuric acid and shake. The appearance of a violet color in the chloroform obviously cannot be accepted as positive proof that the iodine came from iodoform, since alkaline iodides and many iodoform substitutes containing iodine, when used in the treatment of wounds, contribute iodine to the urine.

NITROBENZENE

Nitrobenzene, $C_6H_5NO_2$, is a yellowish liquid having a high index of refraction and the odor of bitter almonds. The boiling-point at 760 mm is 209° . In dilute aqueous solution it has a pronounced sweet taste. Nitrobenzene is easily volatile with steam.

Preparation.—Gradually introduce benzene in small quantities into a mixture of concentrated sulphuric and nitric acid (sp gr 1.41 = 67.5 per cent) so that the temperature of the mixture does not exceed 35° . As soon as all the benzene has been added, shake frequently and warm gently for about 1 hour upon the water-bath. Finally pour the product of the reaction into water.



Toxic Action.—Nitrobenzene has a strong poisonous action. Administration of very small quantities of this compound has produced death in human beings. There are records in the literature of several cases where 20 drops, and even 7-8 drops, have caused fatal results. But on the other hand complete recovery has followed poisoning by much larger doses. Fatal poisonings have come also from inhaling nitrobenzene vapor. Within recent years nitrobenzene has been used to some extent as an abortifacient. Nitrobenzene poisons the blood and changes its appearance. It has a chocolate color and at the same time the red blood-corpuscles change their shape and pass into solution. As a result the blood is incapable of uniting with oxygen. The blood of persons poisoned by nitrobenzene is said to contain less than 1 per cent of oxygen so that death is caused by asphyxiation. Healthy blood contains about 17 per cent of oxygen by volume. There seems to be no methaemoglobin in blood containing nitrobenzene. Such blood examined spectroscopically shows the two oxyhaemoglobin bands and also a special absorption-band between C and D (Fihlens's nitrobenzene band). It is probable that the slight solubility of this poison necessitates a definite incubation period, for 2-3 hours usually elapse after nitrobenzene has been taken before signs of intoxication appear. A woman, who had taken 10 drops of turpentine oil as an abortifacient, gave no indication of intoxication, that is, unconsciousness and cyanosis, for 8 hours after taking the poison.

Some nitrobenzene passes into the urine. Apparently the organism does not convert it into aniline. In nitrobenzene poisoning human urine contains a brown pigment but only rarely haemoglobin or methaemoglobin. Urine containing nitrobenzene will reduce Fehling's solution. It is also fermentable and distinctly laevo-rotatory. Possibly it contains a conjugated glycuronic acid.

Behavior of Nitrobenzene in the Cadaver

In a case of nitrobenzene poisoning Rossi¹ showed that the characteristic odor of nitrobenzene can be detected in the cadaver for only a few days. After about 10 days it entirely disappears so that nitrobenzene can no longer be detected as such. Instead of attempting to find nitrobenzene, the analyst should turn his attention to aniline, the reduction product of nitrobenzene.

Detection of Nitrobenzene

In nitrobenzene poisoning the urine and all the organs have the odor of this compound. For the chemical tests the material should first be distilled with water. Nitrobenzene distils quite easily with steam and appears in the distillate as yellowish globules. These are heavier than water and have a characteristic odor. To detect nitrobenzene with greater certainty, reduce it to aniline and test for that. For this purpose vigorously agitate the globules, separated as completely as possible from water, with granulated tin and a few cc. of

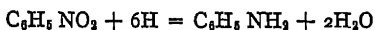
¹ A. Rossi: Changes in Nitrobenzene during Cadaveric Putrefaction. *Boll. Chim. Farm.* 53 (1914), 65

concentrated hydrochloric acid until there is no odor of nitrobenzene. Pour the acid solution from undissolved tin and add an excess of potassium hydroxide solution to decompose the double chloride of aniline and tin. Extract free aniline with ether. Separate the ether solution and evaporate or distil. Dissolve the residual oil-drops by agitation with water and use this solution for the hypochlorite and phenyl-isocyanide test (see page 72).

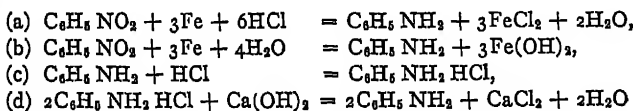
ANILINE

Aniline, $C_6H_5NH_2$, is a colorless, oily, highly refracting liquid having a characteristic aromatic odor and a burning taste. Exposed to air it soon takes on a yellow to brown color and in the end is completely resnified. It boils at 183° . Aniline is only slightly soluble in water (1:31) but mixes in every proportion with alcohol, ether, benzene, chloroform and carbon disulphide. It is without action upon litmus paper. In aqueous solution aniline salts exhibit an acid reaction.

Preparation.—Aniline is prepared by reducing nitrobenzene by means of nascent hydrogen



Place in a cast-iron pan provided with a stirrer equal quantities by weight of nitrobenzene and water. Then gradually add iron turnings and concentrated hydrochloric acid. In the technical process a smaller quantity of hydrochloric acid is sufficient to bring about complete reduction, since the ferrous chloride first formed acts as a carrier of hydrogen, imparting to the metallic iron the property of reducing nitrobenzene to aniline in presence of water. Aniline is contained in the reaction-mixture partly as the hydrochloride. The latter is decomposed by calcium hydroxide and free aniline is then distilled off by means of steam.



Toxic Action.—Aniline is moderately toxic in its action. Doses of 1.5–2 grams, administered in the course of a day, have proved fatal to small dogs. It is not possible to state definitely the average lethal dose for human beings. Very serious results are said to have followed a dose of 3–4 grams of aniline. The lethal dose is certainly less than 25 grams, for that quantity of aniline was sufficient to kill a healthy man. Even inhalation of aniline vapor may cause severe or fatal intoxications.

Aniline produces methaemoglobin and therefore poisons the blood. The conversion of oxyhaemoglobin into methaemoglobin by aniline may be demonstrated by adding an aqueous aniline solution to blood in a test-tube. Aniline

changes their form and partially decomposes red blood-corpuscles. Thereby the quantity of available oxygen in the blood is so diminished that it amounts to only 5-10 volumes instead of 15-20, the normal quantity. The number of red blood-corpuscles is diminished in aniline poisoning but not that of the white blood-cells.

R v Engelhardt¹ has shown that aniline is partly changed in the human organism into aniline black, or into a similar compound insoluble in water. At the climax of aniline poisoning blue-black granules may be seen in every drop of blood and also in the urine. The organism rids itself of aniline by oxidizing it to p-amino-phenol, $\text{HO C}_6\text{H}_4 \text{NH}_2$ (1, 4), which like all phenols combines with sulphuric acid to form an ethereal sulphuric acid, that is, p-amino-phenyl-sulphuric acid, $\text{HO SO}_2 \text{O C}_6\text{H}_4 \text{NH}_2$ (1, 4). The latter in the form of an alkali salt is eliminated through the kidneys and then appears in the urine. A part of the p-amino-phenol is also eliminated as a conjugate of glycuronic acid. The reduction of Fehling's solution by urine containing aniline is due to this conjugated acid. In severe cases of poisoning, unchanged aniline has also been found in the urine. Usually urine that contains aniline has a very dark color. In addition to the substances mentioned, a dark pigment has been detected in urine in aniline poisoning, as well as haemoglobin, methaemoglobin and an abundance of urobilin.

Detection of Aniline

Aniline is a rather feeble base and part of it will pass over with steam, when a solution acidified with tartaric acid is distilled. To estimate aniline quantitatively in any material, distillation should be as complete as possible. Mix the substance with water, make strongly alkaline with sodium hydroxide or carbonate solution, and distil in a current of steam. Since 30 parts of water at 15° dissolve 1 part of aniline, a considerable amount of this base may remain in solution in the distillate. When the quantity is large, oil-drops will appear. An aqueous aniline solution (aniline water) colors pine wood and elder pith intensely yellow. The following tests for aniline should be applied to the distillate.

1. Hypochlorite Test.—Add a few drops of aqueous calcium or sodium hypochlorite solution drop by drop to a portion of the distillate. A violet-blue, or more of a purple-violet color, gradually changing to a dirty red, will appear if aniline is present. Addition of a little dilute aqueous phenol solution containing some ammonia will produce a blue color that is quite stable. This test is sensitive 1:66,000.

2. Phenyl-isocyanide Test.—Heat a portion of the distillate with a few drops of chloroform and potassium hydroxide solution. The repulsive odor of phenyl-isocyanide will show the presence of aniline.

¹ Contributions to the Toxicology of Aniline. Franz Diss Dorpat 1888

3. **Bromine Water Test.**—This reagent added to a solution containing aniline will produce a flesh-colored precipitate. This test is sensitive 1:66,000

4. **Chromic Acid Test.**—Rub in a porcelain dish a trace of pure, absolute aniline with 4-5 drops of concentrated sulphuric acid and add a drop of an aqueous potassium dichromate solution. After a few minutes, the mixture beginning at the edge will take on a pure blue color. Addition of 1-2 drops of water will produce at once a deep blue color. To apply this test to the distillate, first extract with ether, evaporate the ether solution, and test an oily residue as described.

ALCOHOL¹

Alcohol, C_2H_5OH , brought into contact with many different parts of the organism is very rapidly absorbed, but especially easily from an empty stomach. Although there is practically no absorption of non-volatile aqueous liquids from the stomach, alcohol is freely absorbed. After absorption it passes into the blood and is then distributed to all organs (see chloral hydrate). Experiments upon dogs, colts and adult horses have shown that blood at the climax of narcosis contains 0.72 per cent of alcohol. There is stupor even when 0.12 per cent. is present. There is difference of opinion among toxicologists with regard to acute alcoholic intoxication, as to whether the poison is uniformly distributed throughout the body, or accumulated in the brain in larger quantity than in other organs. The following percentages of alcohol, found in the organs of a man who had died at the climax of acute alcohol poisoning, lend support to the latter view: liver 0.21, brain 0.47 and blood 0.33 per cent. These figures become intelligible only when it is assumed that alcohol is combined in the brain. But red blood-corpuscles combine with alcohol as they do with other narcotics.

Uncertainty with regard to the subsequent fate of alcohol in the organism has finally been removed as the result of a series of thorough investigations. These experiments have shown that alcohol is never eliminated unchanged through the skin. At most only 1-1.5 per cent. passes off through the kidneys and only 1.6-2 per cent. through the lungs. Strassmann² found the quantity eliminated by

¹ In this book the word "alcohol," unqualified by an adjective, such as methyl, amyl, etc., means ethyl alcohol. Tr

² Pfüger's Archiv 49 (1891), 315

the lungs somewhat higher (5-6 per cent) and by the kidneys 1-2.5 per cent. The remainder is completely oxidized in the human organism to carbon dioxide and water.

Distribution of Alcohol in Alcohol Poisoning

B. Fischer found the following quantities of alcohol in organs removed from a man who had probably died from drinking too much brandy.

Weight	Organ	Alcohol
2720 grams	Contents of stomach and intestines	30.6 grams
2070 grams	Heart, lungs and blood	10.9 grams
1820 grams	Kidneys and liver	7.8 grams
1365 grams	Brain	4.8 grams

In the internal organs of the cadaver of a man who had died without doubt as the result of acute alcohol poisoning from brandy, A. Juckenack¹ found the following percentages of alcohol: blood from the heart and large blood-vessels 0.53, parts of the heart, lungs, omentum and spleen 0.44, stomach and oesophagus together with contents 0.72, kidneys 0.37, urine 0.65, liver and gall-bladder 0.24, and brain 0.42 per cent.

Detection of Alcohol

Alcohol distils easily with steam and consequently most of it will be in the first fraction. If present in sufficient quantity, it can be recognized in the distillate by its odor. The following tests should be applied to the distillate.

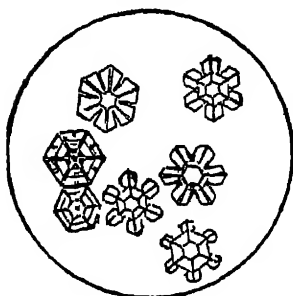


FIG. 12.—Iodoform crystals, from 0.1 gram of alcohol in 5 cc. of a mixture of water, iodo-potassium iodide and potassium hydroxide

1. **Lieben's Iodoform Test.**²—Gently warm the liquid (40-50°), add a few cc. of aqueous iodo-potassium iodide solution, or a small crystal of iodine, and enough potassium hydroxide solution to give the liquid a distinct yellow to faint brownish color. If alcohol is present, a yellowish white to lemon-yellow precipitate of iodoform will appear immediately, or as the solution cools. If the quantity of alcohol is very

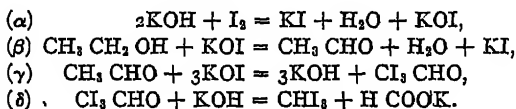
small, a precipitate will form upon long standing. When iodoform is deposited slowly, the crystals are very perfect hexagonal plates and stars (Fig. 12).

¹ A. Juckenack: Contribution to the Chapter "Alcohol Poisoning" *Zeitschr. f. Unters. d. Nahrung- u. Genussm.* 16 (1908), 732

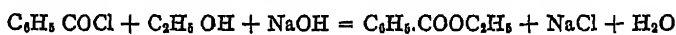
² A. Lieben. Formation of Iodoform and Use of this Reaction in Chemical Analysis *Annalen d. Chem. Suppl.* 7 (1870), 218

Notes.—Lieben's iodoform test is very delicate but not characteristic of alcohol. Other primary alcohols, except methyl alcohol, and many secondary alcohols, as well as aldehydes, ketones, acetic ether, aceto-acetic ester, lactic acid, etc., also give iodoform with iodine and potassium hydroxide or alkaline carbonate.

The correct explanation of the iodoform reaction is probably the following. Iodine and potassium hydroxide form potassium hypo-iodite (KOI) by equation (α). This compound brings about the oxidation of alcohol to acetic aldehyde (β) and at the same time substitutes iodine for hydrogen in the latter (γ). Finally tri-iodo-acetic aldehyde is decomposed by the excess of potassium hydroxide into iodoform and potassium formate (δ).



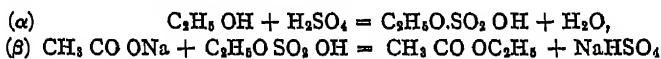
2. Berthelot's Test.—Shake the distillate with a few drops of benzoyl chloride, $\text{C}_6\text{H}_5\text{COCl}$, and an excess of 10 per cent. sodium hydroxide solution until the irritating odor of benzoyl chloride has gone. The aromatic odor of ethyl benzoate will appear. Ten cc. of 0.5 per cent. alcohol will give a distinct odor of this ester:



3. Chromic Acid Test.—Warm the liquid containing alcohol with dilute sulphuric or hydrochloric acid, and add 1–2 drops of very dilute potassium dichromate solution. The color of the liquid will change from red to green, and at the same time the odor of acetaldehyde will be recognized. This test is not characteristic of alcohol because many other volatile organic compounds behave similarly.



4. Ethyl Acetate Test.—Mix the liquid containing alcohol with the same volume of concentrated sulphuric acid. Add a very small quantity of anhydrous sodium acetate and heat. Ethyl acetate will be recognized by its odor:



5. Vitali's Test.—Thoroughly mix a few cc. of distillate in a glass dish with a small piece of solid potassium hydroxide and 2–3 drops of carbon disulphide. Let this mixture stand for a short time without warming. When most of the carbon disulphide has evaporated, add a drop of ammonium molybdate solution (1:10) and then an excess of dilute sulphuric acid. If the distillate contains alcohol,

a red color will appear. Potassium xanthogenate, $\text{SC}(\text{OC}_2\text{H}_5)(\text{SK})$, is first formed. This compound gives a red color with ammonium molybdate. This test is given distinctly by 5 per cent alcohol. Acetone and acetaldehyde produce a similar color.

ACETONE

Acetone, $(\text{CH}_3)_2\text{CO}$, is a colorless liquid of characteristic odor, boiling at 56° and miscible in every proportion with water, alcohol, ether and chloroform. Human urine almost always contains a very small quantity of acetone, about 0.05 gram daily, as a physiological constituent. Under pathological conditions, especially in diabetes mellitus (diabetic acetonuria), urine contains much more. It is also present in urine in prolonged high fever, digestive disturbances, severe forms of carcinoma (carcinomatous acetonuria), etc. Finally acetone has been found in urine in considerable quantity in various intoxications (toxic acetonuria), for example, in poisoning by phosphorus, carbon monoxide, atropine, curare, antipyrine, pyrodine, sulphuric acid, extract of male fern; in chronic lead poisoning; and in chronic morphinism after discontinuance of the drug.

Drugs causing an increase in the formation of acetone in man are benzene, salol, benzonaphthol and heroine (diacetyl-morphine) (R. Kobert "Intoxikationen").

Acetone is not poisonous nor in the least corrosive. Man and animals can tolerate considerable quantities of acetone taken internally. It seems to produce no effect, though it may possibly possess very feeble narcotic properties. Archangelsky found that dogs show signs of narcosis when the blood contains 0.5 per cent of acetone. Even smaller doses produce narcosis in rabbits and have an injurious action upon the blood and kidneys.

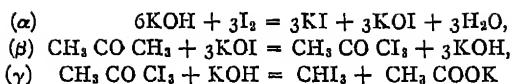
Distillates from human urine, as well as from blood and various organs, such as liver, spleen, kidneys, brain, etc., often contain acetone, or more correctly perhaps, substances like acetone. This is especially the case when cadaveric material has begun to putrefy.

Detection of Acetone

Acetone distils easily with steam and therefore is found in the first fraction of the distillate. This should be used for the following tests.

1. **Lieben's Iodoform Test.**—Add a few cc of aqueous iodo-potassium iodide solution, or a small crystal of iodine, to an aqueous solution of acetone and then potassium hydroxide solution drop by drop until the color is faint yellow. Iodoform immediately separates, even in the cold, as a yellowish white precipitate which is usually amorphous. Acetone differs from alcohol in giving iodoform, when ammonium hydroxide solution is substituted for potassium or sodium hydroxide solution (Gunning's acetone test). Acetaldehyde resembles acetone in giving iodoform in the cold and under conditions the same as those stated above.

Note.—Potassium hypo-iodite (α) probably converts acetone into tri-iodo-acetone, CH_3COCl_3 (β), and this compound is then decomposed by potassium hydroxide into iodoform and potassium acetate (γ).



2. **Legal's Test.**—Add a few drops of freshly prepared sodium nitroprusside solution to a liquid containing acetone, and then potassium hydroxide solution. A red or reddish yellow color will appear. This color soon changes to yellow. Add an excess of acetic acid to the solution. The solution will now have a carmine to purplish red color, according to the quantity of acetone present. Heat will change this color to violet.

This test is given by acetaldehyde but not by alcohol. The red color caused by aldehyde fades upon addition of acetic acid and changes to green with heat. Le Nobel states that ammonium hydroxide, or ammonium carbonate solution, may be substituted for potassium hydroxide solution in Legal's test, but under these conditions the red color is very slow to appear. Le Nobel's modification, however, eliminates the possibility of confusing acetone with acetaldehyde.

3. **Penzoldt's Test.**—Prepare a hot, saturated, aqueous solution of o-nitrobenzaldehyde, $\text{C}_6\text{H}_4(\text{NO}_2)\text{CHO}$ (1, 2), and allow it to cool. Add this solution to the liquid containing acetone and also some sodium hydroxide solution. At first the color of the mixture is yellow. It then becomes green and a blue precipitate of indigotine is formed in 10–15 minutes. When indigotine is present in traces only, shake the solution with chloroform. This solvent will dissolve the coloring-matter and become blue.

4. **Reynold's Test.**—Acetone will dissolve freshly precipitated mercuric oxide and this test is based upon this property. Add mercuric chloride solution to the distillate and an alcoholic potassium hydroxide solution. Shake thoroughly and filter. Add ammonium sulphide solution to the clear filtrate as an upper layer. If acetone is present, there will be a black zone (HgS) where the two solutions meet. Use a double filter for filtering the excess of mercuric oxide and continue pouring the filtrate through the paper until it is perfectly clear. According to v. Jaksch, acetaldehyde also dissolves mercuric oxide without reduction which has been confirmed by Salkowski.

Detection of Acetone in Urine

Acidify 200–500 cc of urine with a few drops of dilute sulphuric acid and distil from a capacious flask. Collect 20–30 cc of distillate. This will contain almost the entire quantity of acetone in the urine. Acetone thus obtained may possibly be derived from aceto-acetic acid often present in human urine, especially in a severe case of *diabetes mellitus*. Distillation decomposes this acid into acetone and carbon dioxide.



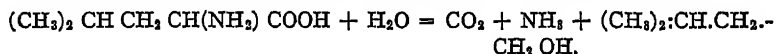
Detection of Alcohol and Acetone in Mixtures

Alcohol may be detected in presence of acetone by the benzoyl chloride reaction. On the other hand, acetone may be distinguished from ethyl alcohol by Legal's or Penzoldt's test.

ISOAMYL ALCOHOL

Isoamyl alcohol, usually known as amyl alcohol, has the formula, $(\text{CH}_3)_2 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$, and is the chief component of the so-called fusel oil. The latter formed together with alcohol during alcoholic fermentation imparts to brandy its unpleasant fusel odor and consists of a mixture of higher alcohols. But fusel oil also contains other substances, for example, furfural. The vapors of amyl alcohol, to which the chemist in his analytical work must occasionally expose himself, are very unpleasant. They may give rise to headache, palpitation of the heart, a feeling of pressure upon the chest, and coughing. Following large doses of amyl alcohol, severe intoxications may appear, manifesting themselves in suspension of respiration, unconsciousness and cyanosis. The presence of alcohol is said to increase its toxicity, for its absorption is made easier. A content of 0.3 per cent of amyl alcohol in alcohol to be used for drinking purposes should be regarded as inadmissible. A part of the amyl alcohol taken into the system is eliminated as such through the kidneys in the urine.

Amyl alcohol is derived from leucine as a result of yeast fermentation



The formation of fusel oil during alcoholic fermentation is attributed to this reaction

Detection of Isoamyl Alcohol

More than traces of amyl alcohol can be recognized in material by its specific odor. To detect amyl alcohol with greater certainty, acidify the material and distill with steam. Extract the distillate with chloroform and evaporate the latter at as low a temperature as possible. Amyl alcohol will appear in the form of oil-drops having a characteristic odor. In case the material available is not too small, prepare a saturated solution by shaking a portion of the oil-drops with water at 15–16°. If this solution is warmed to about 50°, it will become milky, provided amyl alcohol is present. Amyl alcohol dissolves at 13.5° in 39 parts of water and at 16.5° in 50 parts. In addition, use the residue from the chloroform solution for the following tests:

1. **Marquardt's Test.**¹—Put the residue into a test-tube with a little water and add a few drops of dilute sulphuric acid. Then gradually add sufficient potassium permanganate solution (1:1000) to give the mixture a red color after 24 hours. Allow the test-tube to stand corked, adding more permanganate solution, if decolorization takes place within the stated time, and test the odor frequently. If the material contains amyl alcohol, as the tube is opened from time to time there will be detected in succession the odors of 1-valerianic aldehyde, i-amylester of 1-valerianic acid, and finally after about 24 hours 1-valerianic acid. The odor of this acid will appear more marked, if the test-tube is gently warmed in the water-bath. Even when the quantity of fusel oil is very small, this odor is so powerful that it is not masked by many foreign odors.

¹L. Marquardt. Quantitative Estimation of Fusel Oil in Brandy. Ber. d. Deutsch. chem. Ges. 15 (1882), 1370, 1661.

2. **Uffelmann's Test.**¹—Add to the oil-drops left by evaporation of the chloroform extract a freshly prepared solution of methyl violet colored green by hydrochloric acid. In presence of 1-amy! alcohol the drops are colored violet or more reddish blue.

Detection of Amyl Alcohol in Alcohol and Brandy

Dilute 40 cc of brandy to 12-15 per cent with water, that is, to about 500 c, and then extract with 15 cc of pure chloroform. Separate the chloroform layer in a separating-funnel and shake it first with the same volume of water. Separate the chloroform from the water and evaporate in a porcelain dish. Use the residue for Marquardt's and Uffelmann's fusel oil tests.

FORMALDEHYDE

Formaldehyde is an aqueous solution containing at least 35 per cent of CH_2O and known by its official name "*Formaldehydum Solutum*." This solution is also frequently called "*Formalin*" or "*Formol*." It is a colorless, aqueous liquid having a pungent odor and containing variable quantities of methyl alcohol. Litmus paper is not affected, or at most faintly reddened by it. It is miscible in every proportion with water and alcohol but not with ether.

Physiological Action.—Undiluted formaldehyde solution acts upon animal skin producing a leather-like formation. Even a 3-4 per cent solution makes the skin rough, hardening the outer layers and gradually killing them. Therefore formaldehyde solutions have the property of hardening animal tissues by coagulating albumin and giving rise to other chemical changes. In this way it prevents them from undergoing putrefaction. These results are sometimes designated as "formalin tanning." Foodstuff, containing protein that has been changed chemically by addition of formaldehyde, partly loses its digestibility and then has less nutritive value. On this account addition of formaldehyde solution to preserve foodstuffs, such as meat, preserved meat, milk, cheese, etc., and also because of the toxicity of formaldehyde is forbidden by law. Even very dilute formaldehyde solutions taken internally bring about hardening, causing changes in the albumin, and killing the cells of the mucous lining of the mouth, stomach and intestines. Official undiluted formaldehyde solution has a caustic action and administered internally may produce fatal results in the course of a few hours. The author has examined the stomach and contents as well as parts of the intestines of a man who by mistake drank half a glass (70-80 cc) of formaldehyde solution, thinking it was fruit-juice. Three hours after the fatal draught he died in the most terrible agony. The urine, blood and internal organs were not submitted for examination. An abundance of formaldehyde was obtained when the material acidified with dilute sulphuric acid was distilled. In this investigation the observation was made that formaldehyde distils very slowly with steam from parts of the cadaver. It was necessary to use a paraffine-bath, to replace the water frequently, and to carry distillation nearly to dryness before even a

¹ Uffelmann. Archiv der Hygiene 1886, 229.

faint test for aldehyde was obtained. Formaldehyde is very resistant toward the putrefactive changes taking place in the cadaver. The author has diluted with water portions of the stomach and intestines, in which formaldehyde had been found, and allowed them to stand for two years in presence of insufficient air. He has then obtained distinct tests for formaldehyde in the distillate from this putrid mass after it had been acidified with dilute sulphuric acid. Even in a rapid current of steam formaldehyde distils very slowly.

Detection of Formaldehyde

1. General Aldehyde Reactions.—Formaldehyde exhibits the powerful reducing action of aldehydes, especially upon various metallic oxides. It causes the gradual deposition of metallic silver from silver nitrate solution after addition of ammonia, and decolorizes an alkaline copper tartrate solution (Fehling's solution) upon application of heat with separation of red cuprous oxide. Formaldehyde also produces a gray precipitate of metallic mercury with Nessler's reagent. If, however, a small quantity of very dilute formaldehyde solution is added to Nessler's reagent, a brownish color first appears and then quickly gives place to a gray turbidity. Acetaldehyde gives a brown to gray-brown precipitate with Nessler's reagent.

2. Reactions with Higher Phenols.—The particular phenols in these reactions are phloroglucinol and resorcinol. A. v. Baeyer¹ found that formaldehyde forms insoluble condensation-products with univalent and polyvalent phenols when they are warmed in aqueous solution with hydrochloric acid.

(a) **Phloroglucinol Test.**—As a result of a study of the reaction with phloroglucinol, Counciler² found that it soon gave a turbidity even with very dilute formaldehyde solutions and hydrochloric acid. Gradually this became denser and changed to thick flocks of undetermined composition. This is a very delicate test for formaldehyde.

(α) **Procedure in Acid Solution.**—Gradually heat to boiling the solution to be tested for formaldehyde with a mixture consisting of equal volumes of hydrochloric acid (sp. gr. 1.19 = 37.5 per cent.) and water and sprinkle upon it a little phloroglucinol. After all the phloroglucinol is dissolved, if formaldehyde is present, turbidity and separation of yellow-red flocks appear. In addition to formalde-

¹ A. v. Baeyer: *Compounds of Aldehydes with Phenols and Aromatic Hydrocarbons*. Ber. d. Deutsch. chem. Ges. 5 (1872), 1094.

² C. Counciler: *Products of the Condensation of Phloroglucinol with Sugars and Aldehydes*. Chem.-Ztg. 20 (1896), 585.

hyde, all other compounds containing methylene (CH_2) give this test. But if formaldehyde or other methylene-compounds are not present, this test gives at most a faint yellow color.

(β) **Procedure in Alkaline Solution.**—This test may also be carried out in alkaline solution. Mix 2 cc. of a 0.1 per cent phloroglucinol solution with 1 cc. of potassium hydroxide solution and add the liquid to be tested for formaldehyde. The appearance of a distinct red color shows presence of formaldehyde. This color test is given only by more dilute formaldehyde solutions, stronger solutions giving no color. A 3 per cent. solution gives only a raspberry-red color, whereas a 0.0004–0.5 per cent. solution changes to a very deep red. To detect formaldehyde in milk, add to 10 cc. 1–2 cc. of a 0.1 per cent. phloroglucinol solution and a few drops of potassium hydroxide solution (L. Vanino¹)

(b) **Resorcinol Test.**—To detect formaldehyde, Lebbin² uses an aqueous solution of 5 per cent. resorcinol and 40–50 per cent. sodium hydroxide solution, mixing equal volumes of this solution and the aqueous solution to be tested, heating to boiling and keeping at that temperature for about half a minute. Even traces of formaldehyde produce a distinct red color.

Notes.—All proteins, albumoses and peptones, interfere with this reaction, for in their presence only relatively large quantities of formaldehyde can be detected by means of alkaline resorcinol solution. In the case of a substance like milk, formaldehyde must first be distilled off and the distillate used for the test. Urine should first be freed from color by means of bone-black and the resorcinol test for formaldehyde then made with the decolorized urine. Another fact to be borne in mind is that alkaline resorcinol solution produces a color like that of the formaldehyde test with chloroform and those compounds, such as chloral hydrate, that give chloroform with caustic alkalis. The limit of delicacy of the chloroform test with alkaline resorcinol solution is 1:5000 but 1:10,000,000 in case of the formaldehyde test. Therefore in making the resorcinol test the liquid under examination should be considerably diluted when chloroform, or substances that yield chloroform with caustic alkalis, are present.

3. **Hehner's Test³ Modified by Leonard.**⁴—Even in high dilution formaldehyde produces a blue-violet color when brought in contact with protein and hydrochloric or sulphuric acid containing ferric chloride.

¹ L. Vanino: Detection of Formaldehyde by Means of Phloroglucinol. *Pharm. Zentralbl.* 40 (1899), 101.

² Lebbin: Detection of Formaldehyde. *Pharm. Ztg.* 42 (1897), 18.

³ O. Hehner: Detection of Formalin. *The Analyst* 21 (1896), 94.

⁴ N. Leonard: Hehner's Formaldehyde Test. *The Analyst* 21 (1896), 157.

Procedure.—Mix in a large test-tube 5 cc of the liquid to be tested for formaldehyde with 2 cc of fresh unboiled milk and 7 cc of hydrochloric acid (sp gr 1.124 = 25 per cent), containing in 100 cc. 0.2 cc. of 10 per cent. ferric chloride solution, and boil gently for about 1 minute. In presence of formaldehyde a violet color will appear.

Notes.—In making this test it must first be established experimentally that the milk used is free from formaldehyde and that it gives the test with a trace of formaldehyde. According to Juckenack, Hehner's test for formaldehyde is sharper than that with morphine-sulphuric acid. Its sensitiveness, however, is strongly influenced when acetaldehyde is present at the same time, especially if the quantity of this aldehyde is quite large. Hehner's test may also be carried out in the manner of a zone-test by carefully adding to pure concentrated sulphuric acid as an upper layer a mixture of 5 cc of the aqueous liquid to be tested with 5 cc of fresh milk and a few drops of very dilute ferric chloride solution. If formaldehyde is present, a violet-blue zone will at once appear at the line of contact of the two layers and remain distinctly visible for 2-3 days. In examining milk for formaldehyde by Hehner's test, dilute it with an equal volume of water and add this mixture as an upper layer to concentrated sulphuric acid, containing a trace of ferric chloride. Milk free from formalin gives a greenish color but a violet-blue ring when it is present.

According to E. Salkowski,¹ any albumose, particularly Witte's peptone, may be substituted for milk in Hehner's test with equally good results. Dissolve by shaking and warming a little Witte's peptone in 8 cc of the liquid to be tested. Then add 3-4 drops of 3 per cent ferric chloride solution, about half the volume of hydrochloric acid (sp. gr 1.19) and heat to boiling. In presence of formaldehyde, a gradually increasing full violet color at once appears and later passes into more of a blue. Hydrogen peroxide may also be substituted for ferric chloride but in excess it destroys the coloring-matter.

Chemical Explanation of Hehner's Formaldehyde Test with Milk

Pure formaldehyde and pure concentrated sulphuric or hydrochloric acid give no color with proteins. For the development of the color-reaction a small quantity of an oxidizing agent is required. In presence of a trace of ferric chloride, potassium nitrite, platonic chloride, sodium peroxide, hydrogen peroxide, sodium percarbonate, ammonium and potassium persulphate, the purple-violet color at once appears. This formaldehyde reaction is characteristic of proteins and is dependent upon the presence of a tryptophane group in the protein molecule. Its intensity varies with different proteins in accordance with the amount of tryptophane they contain. For this reason gelatine does not give this reaction. The certainty with which colors appear in presence of tryptophane, indole and skatole, renders solutions of these substances suitable for the colorimetric estimation of small quantities of formaldehyde.

The test for formaldehyde in milk first described by Hehner is not a general aldehyde reaction but apparently is confined to formaldehyde alone. Moreover

¹ E. Salkowski. Contribution to the Knowledge of Some Formaldehyde Reactions. *Biochem Zeitschr* 68 (1914), 337.

most aromatic hydrocarbons and acids as well as phenols and amino-compounds give no similar color

To detect formaldehyde in milk, the latter is diluted ten times. This test is more delicate, if half of a mixture of 50 cc. of milk and 20 per cent phosphoric acid is distilled off. According to v. Fillinger¹ even 0.004 per cent of formaldehyde can be detected direct in milk but 0.0008 per cent in the distillate. To detect formaldehyde in butter and meat, melt the former in presence of water under a reflux and then test the aqueous solution, mix the meat with the same weight of 20 per cent. phosphoric acid and then distil off half the total weight. In this way even 0.024 per cent of formaldehyde can be detected in meat with certainty, with 0.016 per cent the reaction was considerably weaker

4. Jorissen's² Morphine-Sulphuric Acid Test.—With a solution of morphine hydrochloride in concentrated sulphuric acid, formaldehyde gives a red-violet to blue-violet color that increases in intensity with time, is quite stable and finally passes into indigo-blue.

Procedure.—Before making the test dissolve 0.05 gram of morphine hydrochloride in 2.5 cc. of concentrated sulphuric acid. Also carefully mix with cooling 1 cc. of the aqueous liquid to be tested with 5 cc. of concentrated sulphuric acid. Cool to room temperature and add the morphine-sulphuric acid solution previously prepared. If formaldehyde is present, the mixture will at once take on a purple-red color.

This test may also be made by dissolving in a porcelain dish a crystal of morphine hydrochloride in about 10 drops of dilute sulphuric acid (1 part of concentrated acid and 6 parts of water). Add to this colorless solution by means of a glass rod a drop of the liquid to be tested. Wirthle makes this test by cooling well and mixing 1 cc. of the aqueous liquid with 5 cc. of concentrated sulphuric acid. He does not use, however, a solution of morphine hydrochloride in 2.5 cc. of sulphuric acid but 0.05 gram of the morphine salt itself.

G. Fendler and C. Mannich³ have compared this test with other formaldehyde tests with regard to sensitiveness. After half a minute a 0.1 per cent formaldehyde solution had a distinct violet color, after 3 minutes a deep dark-violet, in the course of 10 minutes a 0.01 per cent solution was distinctly violet, the color increasing with time; within 15 minutes a 0.005 per cent. formaldehyde solution was distinctly violet, within 1 hour a 0.001 per cent. solution had a barely per-

¹ F. v. Fillinger: Detection of Formaldehyde. *Zeitschr. f. Unters. d. Nahrsg.-u. Genussm.* 16 (1908), 226.

² A. Jorissen: Detection of Formalin in Foods. *Rev. intern. falsific.* 11 (1898), 12

³ G. Fendler and C. Mannich: Detection of Methyl Alcohol in Alcoholic Preparations. *Arb. a. d. Pharm. Institut der Universität Berlin* 3 (1906), 243

ceptible violet color. Therefore even 0.01 milligram of formaldehyde can be detected in a dilution of 1:100,000. The resorcinol test fails even at a dilution of 1:20,000, whereas the phloroglucinol test is equal to the morphine test in delicacy. The latter, however, has the advantage of being characteristic and can also be observed more easily than the very transient phloroglucinol test. Fendler and Mannich consider the morphine-sulphuric acid test as the most serviceable reaction for the detection of formaldehyde. With this morphine-reagent acetaldehyde gives only yellow colors.

5. Fuchsine-Sulphurous Acid Test. (a) According to Denigès.¹—The color given by fuchsine-sulphurous acid with an equal volume of a sufficiently dilute acetaldehyde solution disappears after a few hours, whereas the color produced by this reagent with formaldehyde increases in intensity with time and besides is very stable. This difference between these two aldehydes manifests itself particularly in strong sulphuric acid solution and serves for the detection of traces of formaldehyde in presence of considerable quantities of acetaldehyde. If, for example, 1.2 cc of sulphuric acid (sp. gr. 1.66 = 73.64 per cent.) are added for every 5 cc of an aqueous acetaldehyde solution and fuchsine-sulphurous acid, hardly any color appears after vigorous shaking and prolonged action. But if the acetaldehyde is replaced by a formaldehyde solution, even a trace of the latter produces a blue or more of a blue-violet color. After 5–10 minutes standing, 0.01 milligram of formaldehyde and less can be detected with certainty by a violet color and an absorption-band in the orange. Obviously all formaldehyde derivatives, which like hexamethylene-tetramine (urotropine) yield formaldehyde under the influence of sulphuric acid, as well as all those compounds that like methyl alcohol give formaldehyde when properly treated, respond to this test.

Preparation of Fuchsine-Sulphurous Acid.—Mix a solution of 1 gram of fuchsine in 1 liter of water with 50 cc of saturated sodium bisulphite solution and acidify with 1 cc. of concentrated sulphuric acid.

(b) According to Grosse-Bohle-Fincke.²—This formaldehyde reaction differs from the general aldehyde tests with fuchsine-sulphurous acid in having present considerable free hydrochloric acid. Of all

¹ G. Denigès. Detection of Traces of Formaldehyde in Presence of Acetaldehyde by Means of Fuchsine-Bisulphite. *Compt. rend. de l'Académie des sciences* 150 (1910), 529.

² H. Fincke. Detection of Small Quantities of Formaldehyde and of Some Formaldehyde Compounds by Means of Fuchsine-Sulphurous-Hydrochloric Acid. *Zeitschr. f. Unters. d. Nahrungs- u. Genussm.* 27 (1914), 246.

acids, hydrochloric acid is best adapted for this purpose, for this reaction is characteristic of formaldehyde at higher acid concentration. Under these conditions other aldehydes do not interfere with this formaldehyde test, unless they are present in too large quantity. Other substances of the most varied character are without influence.

Procedure.—Mix 10 cc of the liquid to be tested for formaldehyde with 1–2 cc. of hydrochloric acid (sp gr 1.124 = 25 per cent.) and then add 1 cc of the reagent. According to the amount of formaldehyde present, a permanent blue-violet to red-violet color appears within a few minutes or at latest 12 hours. Obviously other substances such as hexamethylene-tetramine and methylal, that undergo decomposition in acid solution giving formaldehyde, also give this test. A successful result is dependent upon the exact observance of instructions.

Preparation of the Reagent.—Dissolve 1 gram of rosaniline acetate or hydrochloride in about 500 cc of water, add an aqueous solution of 25 grams of crystallized sodium sulphite together with 15 cc of hydrochloric acid (sp gr 1.124 = 25 per cent.), and bring to 1 liter with water.

Detection of Formalin in Milk

(Grosse-Bohle¹)

Add 2 cc of concentrated hydrochloric acid to 10 cc of water, shake and add 1 cc of a fuchsin solution decolorized by means of sulphurous acid, containing 1 gram of pure rosaniline hydrochloride in a liter. Larger amounts of formalin can be recognized after 10–15 minutes, smaller amounts after a few hours, by a red-violet color in the milk. After 12 hours the intensity of color reaches a maximum and may be used for a colorimetric estimation. By this test even 0.005–0.01 cc. of "Formalin" in 1 liter, or 1 drop in 5–10 liters of milk, may be detected.

6. Rimini's² Phenyl-hydrazine Test.—Add 1 cc. of a dilute aqueous solution of phenyl-hydrazine hydrochloride together with a few drops of a freshly prepared solution of sodium nitro-prusside, and finally a few drops of concentrated sodium hydroxide solution to 10–15 cc. of a very dilute formaldehyde solution. A blue color will appear and later pass into red. This blue color can be recognized in milk even in presence of 1/30,000 part of formaldehyde.

¹ H. Grosse-Bohle. The Hygienic Inspection of the Milk Business. Zeitschr. f. Unters. d. Nahrungsm. u. Genussm. 14 (1907), 78.

² E. Rimini: Detection of Formaldehyde in Foods. Ann. di Farm. 3 (1898), 97; Chem. Zentralbl. 1898, I, 1152.

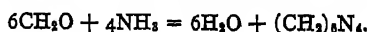
In this reaction ferric chloride in hydrochloric acid solution may be substituted for sodium nitro-prusside in alkaline solution in the following manner. Add in succession to 10 cc of very dilute formaldehyde solution 2 cc of a 4 per cent aqueous solution of phenyl-hydrazine hydrochloride, 6 drops of 10 per cent ferric chloride solution, and 5 cc. of hydrochloric acid. This mixture will have a raspberry-red color. Under the same conditions acetaldehyde gives a light yellow color. These two formaldehyde tests are delicate and according to E Rimini are given by no other aldehyde. They are adapted for the detection of formaldehyde in foods, especially in milk.

According to Meth,¹ the test last described is also given by acrolein but the shade of color is somewhat different and it is less sensitive. In examining fats that have become rancid, to avoid error, Meth employs the very sensitive first test of Rimini with phenyl-hydrazine hydrochloride, sodium nitro-prusside and sodium hydroxide solution. In this case the blue color is produced only by formaldehyde and not by acrolein.

Schryver's² Procedure

Add 2 cc of a freshly prepared and filtered 1 per cent solution of phenyl-hydrazine hydrochloride, then 1 cc of a fresh 5 per cent. potassium ferricyanide solution, and finally 5 cc of concentrated hydrochloric acid to 10 cc of an aqueous solution containing free formaldehyde. A splendid color similar to fuchsine will appear. In the case of combined formaldehyde, for example, hexamethylene-tetramine, warm the mixture for a short time after adding phenyl-hydrazine hydrochloride or allow it to stand, and then add potassium ferricyanide and hydrochloric acid. In presence of other dyestuffs, especially when only small amounts of formaldehyde are present, dilute with water after adding the reagents. Then add ether and shake. The hydrochloride of the chromogenic base undergoes dissociation and the latter is dissolved by the ether. Shake the ether solution with a little concentrated hydrochloric acid which will take up the dyestuff as the colored hydrochloride. Acetaldehyde does not give this test. Furfural gives with this mixture an apricot-yellow color, which becomes dirty green with concentrated hydrochloric acid, is transient, and is not given at all in a concentration of 1:100,000. This test was employed for the detection of formaldehyde synthesized through the agency of chlorophyll.

7. Hexamethylene-tetramine Test.—Formaldehyde easily condenses with ammonia forming hexamethylene-tetramine:



With mercuric chloride and potassium mercuric iodide it gives characteristic crystalline precipitates. Add ammonia repeatedly and evaporate to dryness in a porcelain dish upon the water-bath the liquid to be tested for formaldehyde, for example, the distillate

¹ Meth: Detection of Formaldehyde. Chem.-Ztg 30 (1906), 666.

² S B Schryver. The Photo-Chemical Formation of Formaldehyde in Green Plants. Proc Roy Soc London, Series 82 (1910), 226.

obtained from any kind of material. If more than traces of formaldehyde are present, the residue will contain the characteristic crystals of hexamethylene-tetramine. They may be recognized by dissolving them in 4-5 drops of water, placing a drop of the solution upon a glass slide, and examining under the microscope

(a) Add to one drop a drop of saturated mercuric chloride solution. If hexamethylene-tetramine is present, a crystalline precipitate will immediately appear. In a short time star-shaped crystals having three and more rays will be seen and later perfect octahedrons.

(b) Add to a second drop a drop of potassium mercuric iodide solution and very little dilute hydrochloric acid. If hexamethylene-tetramine is present, light yellow hexagonal stars will form.

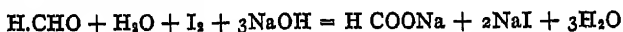
8. Codeine and Sulphuric Acid Test.—Dissolve codeine in a little concentrated sulphuric acid and then add a drop of the liquid to be tested for formaldehyde. An intense blue color will appear if formaldehyde is present. Acetaldehyde and furfural do not give this blue color. An absolutely pure sulphuric acid free from ferric oxide must be used for this test, otherwise an impure acid of that description will itself produce a blue color with codeine. Dilute sulphuric acid may also be substituted for the concentrated acid

Quantitative Estimation of Formaldehyde

Various volumetric methods have been devised for the quantitative estimation of formaldehyde in aqueous solution. Two of these will be described here. Other methods of estimating formaldehyde will be found in Chapter V on pages 548 to 551.

1. Romijn's¹ Iodimetric Method

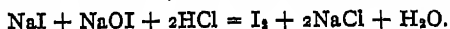
This method is based upon the easy oxidation of formaldehyde to formic acid by iodine in alkaline solution



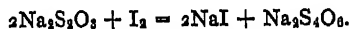
Oxidation is complete within 10 minutes. Iodine must always be present in excess. That not combined with formaldehyde will react with sodium hydroxide:



If the alkaline solution is acidified, iodine will again be set free:



Finally iodine thus liberated is estimated by titration with 0.1 N-sodium thio-sulphate solution.



¹G. Romijn: Estimation of Formaldehyde. *Zeitschr. f. analyt. Chem.* 36 (1897), 18.

Procedure.—Mix in a glass-stoppered flask 10 cc of a formaldehyde solution, diluted to a definite volume, with 25 cc. of 0.1 n-iodine solution and sufficient strong sodium hydroxide solution to produce a light yellow color. Stopper the flask and allow the mixture to stand for 10 minutes protected from light. Then add dilute hydrochloric acid until the color is deep brown and titrate free iodine with 0.1 n-sodium thiosulphate solution, using starch solution as indicator.

Calculation.—According to the equation, 1 molecule of formaldehyde requires 2 atoms of iodine, 1000 cc of 0.1 n-iodine solution, containing 0.1 gram-atomic weight of iodine, correspond therefore to $\frac{\text{CH}_2\text{O}}{20}$ grams = $\frac{30.02}{20} = 1.501$ gram of formaldehyde and 1 cc of 0.1 n-iodine solution corresponds to 0.001501 gram of CH_2O .

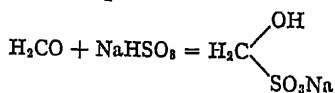
Example—10 cc of very dilute formaldehyde solution were taken. 25 cc of 0.1 n-iodine solution were used. By titration 10.2 cc of 0.1 n-iodine remained uncombined. Therefore the formaldehyde required $25 - 10.2 = 14.8$ cc of 0.1 n-iodine. This volume corresponds to $14.8 \times 0.001501 = 0.0223$ gram of CH_2O contained in the 10 cc of formaldehyde solution taken.

Notes.—Romijn's method is very useful when pure formaldehyde solutions are used. But it is not applicable to solutions that contain other aldehydes, alcohol, acetone, or any substances that will combine with iodine.

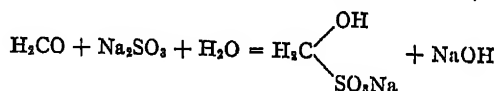
2. Sodium Sulphite Method

(Lemme¹ and Doby²)

Formaldehyde combines very easily with sodium bisulphite to form oxy-methyl-sodium sulphonate



This tendency of formaldehyde to form its bisulphite compound is so strong that even in a neutral solution of sodium sulphite, Na_2SO_3 , the reaction takes place at once and at the same time a corresponding equivalent of sodium hydroxide is set free (G. Lemme):



¹ G. Lemme: Estimation of Formaldehyde in Solutions. Chem. Ztg. 27 (1903), 896.

² G. Doby: Estimation of Commercial Formaldehyde. Zeitschr. f. angew. Chem. 20 (1907), 353.

This free sodium hydroxide is then estimated volumetrically and in that way the amount of formaldehyde in a pure or crude solution can be determined. Since aqueous solutions of sodium sulphite react alkaline to phenol-phthalein, the red color produced by this indicator must first be discharged by addition of a few drops of sodium bisulphite solution. Doby recommends rosolic acid as an indicator, since its behavior toward sodium sulphite solution is entirely neutral

Requirements. 1. 2 n-Sodium Sulphite Solution.—Dissolve 252.3 grams of crystallized sodium sulphite, $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$, in water to 1 liter. This solution does not keep indefinitely, since sulphites owing to external catalytic influences go over into sulphates in presence of air. Therefore the quantity of sodium sulphite in solutions that have been kept for some time should be determined beforehand by titration with 0.1 n-iodine solution.

2. Rosolic Acid Solution.—Dissolve 1 gram of rosolic acid in 40 cc. of 50 per cent alcohol.

Procedure.—If formaldehyde is to be estimated in a commercial formalin solution containing 35–40 per cent, use a solution that has been diluted ten times. According to Doby, 25 cc. of formalin are weighed in a 100 cc. flask and water is added to the mark. Add to 10 cc. of this diluted solution 50 cc. of sodium sulphite solution, previously neutralized with normal acid, and 2 drops of rosolic acid solution. Titrate the solution that is now red with n-hydrochloric acid. According to the above equation, 1 molecule of sodium hydroxide is set free for 1 molecule of formaldehyde. Therefore 1000 cc. of n-acid correspond to a gram-molecular weight = CH_2O grams = 30 grams of formaldehyde and 1 cc. of n-acid corresponds to 0.03 gram of CH_2O .

Notes.—This method is especially well adapted for the estimation of formaldehyde in aqueous solutions containing iron such as collect in the formalin vacuum apparatus. Dissolved iron, which would interfere with the procedure, is converted into a complex compound by addition of neutral Rochelle salt so that no precipitation of iron in the alkaline liquid takes place. If, however, the formaldehyde solution is already turbid owing to separation of iron, phenol-phthalein is to be preferred to rosolic acid as indicator, because it imparts a stronger red color to the alkaline liquid.

METHYL ALCOHOL

Methyl alcohol or wood spirit, CH_3OH , like ethyl alcohol is a colorless liquid of characteristic odor, boiling between 66.2–66.8° and miscible in every proportion with alcohol, acetone, ether and

chloroform Methyl alcohol is an excellent solvent for fats, oils, resins and varnishes and is frequently used instead of alcohol for this purpose.

Physiological Action.—Methyl alcohol like alcohol is intoxicating Taken in larger quantities, especially in concentrated form, it is toxic in its action In its capacity as a solvent of fats methyl alcohol should exert a very strong haemolytic action This, however, is not the case, at least its haemolytic action is not as strong as that of alcohol But it acts as a local irritant poison upon those mucous surfaces with which it comes in contact Owing to its solvent action upon lipoids it exerts its toxic power by paralyzing the central nervous system Finally the gross anatomical changes that it causes in various organs take place more rapidly and are more severe than those produced by alcohol In more serious poisoning by methyl alcohol, when death does not ensue, frequently dangerous derangement of vision results which may end in total blindness These disturbances may appear in a few hours even after taking a dose of methyl alcohol that is not lethal Very frequently cystitis is an after-effect of methyl alcohol poisoning, a possible explanation of which may be that the poison leaves the body in a form irritating to the urinary passage Methyl alcohol, at least in part, is oxidized to formic acid in the animal organism According to experiments made by Autenrieth,¹ a man, who in a period of 8 days had imbibed 80 grams of pure methyl alcohol in a very dilute form, excreted during this time 5.6 grams more formic acid than he had for the same time under normal conditions A remarkable fact in this case was that the maximum amount of formic acid was not found in the urine until the third and fourth day after the last dose of methyl alcohol had been taken Since formic acid is a normal constituent of human urine, obviously a mere qualitative test for its presence does not afford sufficient grounds for coming to a definite conclusion with regard to suspected methyl alcohol poisoning. In such cases a quantitative estimation of formic acid in the urine of the person supposed to have been poisoned by methyl alcohol is absolutely essential. If the formic acid in the urine shows a marked increase, even after entirely harmless quantities of methyl alcohol have been taken, this will be all the more the case in severer poisoning, especially when it terminates fatally. After internal administration of methyl alcohol, formaldehyde can never be found in the urine, but a trace of unchanged alcohol may be detected, that is, when 30 grams of pure methyl alcohol in very dilute form have been taken in the course of 2 days Methyl alcohol is retained in the human body for some time and is only slowly oxidized This fact is proved by the results of Autenrieth's experiments which show that the maximum quantity of formic acid is not eliminated until the third and fourth day after the last dose of methyl alcohol. For this reason methyl alcohol can still be detected in the cadaver even when death does not ensue for a day after the last drink of this alcohol The quantity of formic acid in the urine of the person poisoned will also show a marked increase. In methyl alcohol poisoning the liver is usually enlarged and very fatty. In the

¹ W. Autenrieth. Amount of Formic Acid Normally Present in Human Urine and after Administration of Different Substances. Münch. med. Wochenschr. 1919, No. 31; page 862.

case of the dog, Pohl¹ found that 16.6 per cent was the average normal amount of fat in the liver, whereas in the case of an animal poisoned by methyl alcohol it rose to 37.9 per cent. Following methyl alcohol poisoning, the spleen in man is usually found enlarged.

Detection of Methyl Alcohol

Methyl alcohol differs from alcohol in not forming iodoform with iodine and solutions of alkaline hydroxides or carbonates. Most of the methods for its detection depend upon two different reactions. Either methyl alcohol is oxidized to formaldehyde and this is detected, or it is converted by means of iodine and red phosphorus into methyl iodide, the latter into dimethyl-aniline, and this compound into methyl violet by means of an oxidizing agent. The analogous diethylaniline from alcohol does not give this result. In many instances methyl alcohol has to be detected in presence of alcohol. In such a case the positive detection of methyl alcohol, against which no objection can be raised, is more or less difficult. In toxicological-chemical investigations the alcohol usually has to be distilled off from gastric and intestinal contents, urine, blood or organs. It must be borne in mind in carrying out this operation that methyl alcohol does not distil as easily with steam as might be supposed from its lower boiling-point. Such investigations usually require the detection of small quantities of methyl alcohol in very dilute aqueous solutions, that is, in distillates. Those methods of detection that depend upon the conversion of methyl alcohol into methyl iodide and methyl violet are not applicable for this purpose, although they are very serviceable in the case of pure alcoholic mixtures, for example, in the detection of methyl alcohol in brandies. Those tests too that have been recommended at various times for the direct detection of methyl alcohol in presence of alcohol are also out of the question.

1. Oxidation Tests for Methyl Alcohol.—The oxidation of methyl alcohol to formaldehyde may be brought about in a variety of ways. It may be effected by means of a copper spiral superficially oxidized by having been previously brought to a red heat, by potassium dichromate and sulphuric acid, by potassium permanganate, also in presence of sulphuric acid as well as catalytically. In the absence of alcohol, which may be shown by first making the iodoform test, the detection of methyl alcohol is simpler. Obviously these tests,

¹ J. Pohl. *Arch. f. exper. Path. u. Pharm.* 31 (1895), 286.

when they are positive, only prove the presence of methyl alcohol if formaldehyde as such is absent. Consequently the material should always first be tested for formaldehyde

(a) **With Glowing Copper Spiral.**—Put the liquid to be tested, for example, distillate, in a test-tube. Then introduce a copper spiral heated to bright redness clear to the bottom of the tube for a very short time, about a second. Repeat this operation 5–6 times, that is, until the lower part of the spiral remains coated with copper oxide. Then test the resulting liquid for formaldehyde by means of one of the color-reactions (see page 80). If this aldehyde is present, the liquid will have more or less of a red color with alkaline phloroglucinol solution. Or make Hehner's test in the following manner described by Kahn¹. Mix 5–10 cc. of the oxidized liquid with 2–5 cc. of fresh milk and a drop of dilute ferric chloride solution. Carefully add this mixture as an upper layer to a few cc. of concentrated sulphuric acid. In presence of formaldehyde, a violet-blue color will appear within 3 minutes at the zone of contact of the two liquids. The test with morphine-sulphuric acid may also be made. In the copper spiral test, undiluted ethyl alcohol must previously be diluted with water ten times.

(b) **With Potassium Permanganate.**—This reagent oxidizes methyl alcohol to formaldehyde and alcohol to acetic acid.

(α) **Oxidation According to Juckenack.**—Add to 5–10 cc. of the liquid to be tested for methyl alcohol, for example, the distillate from any material, an equal volume of dilute sulphuric acid and a very small quantity of finely ground potassium permanganate. If a reaction does not take place at once, set the mixture in water at 50°. As soon as the red color has gone, add a little more potassium permanganate, keeping the temperature at about 50°. Proceed in this manner until the red color holds for at least 5 minutes. Then allow the solution to stand in warm water at 50° until the color is gone. Finally filter through a small paper, returning the filtrate to the paper until it is clear and colorless. Potassium permanganate that may be present is reduced by the paper and in this way the solution loses its color. The author does not recommend decolorizing the still red liquid by adding a few drops of dilute alcohol. Test a portion of the colorless filtrate for formaldehyde with milk and hydrochloric acid containing ferric chloride and a second portion with

¹ J. Kahn Detection of Methyl Alcohol in Liquids Containing Alcohol, Pharm Ztg 50 (1905), 655

morphine-sulphuric acid. Make this second test by adding 5 cc. of concentrated sulphuric acid with cooling to 1 cc. of the filtrate and then adding to this cold mixture a freshly prepared solution of 0.05 gram of morphine hydrochloride in 2.5 cc. of concentrated sulphuric acid. The morphine salt itself (0.05 gram) may be added without being first dissolved (Wirthle). The former test is sharper than that with morphine-sulphuric acid but the presence of considerable quantities of acetaldehyde at the same time causes serious interference.

(β) **Oxidation and Denigès' Test.**—According to Denigès, in testing with fuchsine-sulphurous acid for formaldehyde formed by the oxidation of methyl alcohol, the presence of alcohol not only does not interfere but even has a favorable influence, since the formol-acetal, $\text{CH}_2(\text{OC}_2\text{H}_5)_2$, appearing as an intermediate product, reacts especially easily with fuchsine-sulphurous acid. Add to the aqueous liquid to be tested for methyl alcohol (about 6 cc.), which should not contain more than 3–4 per cent. of methyl alcohol, 0.2 cc. of purest alcohol, 5 cc. of 2.5 per cent. potassium permanganate solution, and 0.4 cc. of sulphuric acid. After 2–3 minutes discharge the color with cold saturated oxalic acid solution (about 8 per cent.), adding to the mixture, after it has become madeira-yellow, 1 cc. of sulphuric acid and shaking. As soon as the liquid is colorless, add 5 cc. of fuchsine-sulphurous acid. A violet color will appear at once and, the more methyl alcohol present, the more intense it will be. This color attains its maximum after about an hour. It is still very strong, if 1 per cent. of methyl alcohol is present, and visible with 0.1 per cent.

Fendler and Mannich¹ have observed that formaldehyde alone is completely destroyed in a short time by excess of potassium permanganate in acid solution, but that a detectable quantity of formalin remains if an alcoholic solution of formaldehyde is oxidized by an amount of potassium permanganate insufficient for the complete oxidation of the alcohol.

The oxidation of methyl alcohol to formaldehyde by platinum black according to Peratoner and Tamburello and by sodium persulphate according to Pazienti, is given in Autenrieth's "*Chemical Detection and Estimation of Poisons*" which forms a part of Abderhalden's "*Biological Laboratory Methods*."

¹ G. Fendler and C. Mannich: Detection of Methyl Alcohol in Alcoholic Preparations. Arb. a. d. Pharm. Inst. d. Univers. Berlin 3 (1906), 243.

Detection of Methyl Alcohol in Parts of Cadavers(Juckenack¹)

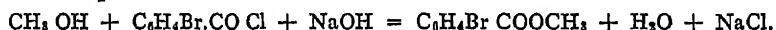
Add to the comminuted cadaveric material 15–20 per cent of sodium chloride, acidify with phosphoric acid, and distil from an oil-bath, the temperature of which is allowed to rise slowly to about 150°, until the weight of the distillate is about half that of the cadaveric material taken. Test the distillate for formaldehyde with milk and ferric chloride-hydrochloric acid according to the procedure described in the “Methods of Procedure of the Meat Inspection Law” (see page 546). If this test is negative, filter from insoluble fatty acids, exactly neutralize the filtrate with sodium hydroxide solution, add 20 per cent sodium chloride, and distil to about one-third. The residue in the distillation flask may be tested as described for formic acid, whereas the distillate may be examined for methyl alcohol (see page 91). One portion of the colorless filtrate finally obtained after oxidation may be tested for formaldehyde with milk and ferric chloride-hydrochloric acid and a second portion with morphine-sulphuric acid. The former reaction is sharper but considerable quantities of acetaldehyde cause interference.

Formic Acid.—Strongly acidify the neutral residue remaining in the distillation flask with phosphoric acid and then distil off about one-third. A reaction given by mercuric chloride with this distillate should not be accepted under all conditions as conclusive proof of the presence of formic acid, because a distillate from animal organs may contain substances that resemble formic acid in their reducing action. The detection of formic acid in these distillates, as carried out by Juckenack, is based upon an observation of Fenton that formic acid in aqueous solution in presence of sulphuric acid is reduced to formaldehyde by magnesium and can be detected by various tests. With this object in view, render the distillate faintly acid with dilute sulphuric acid and, by adding each time magnesium turnings in small amount, keep up an evolution of hydrogen that is not too violent but is always active. When traces of formic acid (about 0.1 per cent.) are present, this action must be maintained for about an hour, whereas larger quantities require only 2–3 minutes. Finally test the filtrate for formaldehyde with milk and ferric chloride-hydrochloric acid.

Notes.—By the method described, Juckenack was able on the occasion of the poisoning epidemic in Berlin asylums (December 1911) to detect methyl alcohol in quite a large number of different parts of many cadavers, whereas the result was negative in the case of putrefying meat, human faeces, and fresh as well as putrid liver. The fresh liver came from a man who had died as the result of an accident. The liver that had passed into putrefaction was also a human liver and was tested as a control. By conducting distillation from an oil-bath in the manner described, the troublesome frothing that occurs in steam distillations as well as over-heating of material was avoided. In the putrefaction of liver sometimes not inconsiderable quantities of ethyl alcohol are produced as a result of the fermentation of glycogen that has undergone cleavage on account of the presence of the blood-ferments.

¹ A. Juckenack and Collaborators: The Berlin Methyl Alcohol Poisonings; Interesting Observations and Experiences from the Practice of the Food-Chemist. *Zeitschr. f. Unters. d. Nahrungsm. u. Genussm.* 24 (1912), 7.

2. **Autenrieth's¹ p-Bromo-benzoylchloride Test.**—In dilute aqueous solution in presence of sodium hydroxide solution, methyl alcohol gives with p-bromo-benzoylchloride the well-crystallized methyl ester of p-bromo-benzoic acid:



On the other hand, alcohol under the same conditions gives the corresponding ethyl ester which remains liquid even at low temperatures. Since p-bromo-benzoylchloride melts at 43° , the liquid to be tested for methyl alcohol should first be heated about to this temperature before the reagent is added.

Procedure.—Add to the aqueous solution to be examined, for example, 40–50 cc. of distillate, about 10 cc. of 10 per cent. sodium hydroxide solution, warm the mixture, best in a glass-stoppered flask, to $40\text{--}45^\circ$, add according to the quantity of methyl alcohol suspected 1–5 grams of ground p-bromo-benzoylchloride, and shake vigorously until cold. The mixture should remain alkaline. From time to time it should be tested with litmus paper. If the reaction becomes acid, further addition of sodium hydroxide solution is necessary. In presence of methyl alcohol, the methyl ester of p-bromo-benzoic acid separates as a white, usually friable powder. It is filtered off, washed with cold water until free from alkali, and dried upon a porous plate or in a vacuum-desiccator for the purpose of finally determining its melting-point ($78\text{--}79^\circ$). After the powder has been washed, crystallize it from alcohol, methyl alcohol or acetone. A solution saturated at $15\text{--}20^\circ$ is prepared, filtered, mixed with an equal volume of water, rapidly stirred and set in ice. From alcohol the methyl ester of p-bromo-benzoic acid is obtained in the form of shining leaflets, from methyl alcohol in fine needles. From hot benzene solution the ester crystallizes upon addition of petroleum ether in shining leaflets. Cold alcohol dissolves it to the extent of 2.3 per cent., but it is more easily soluble in acetone.

Methyl alcohol in aqueous solution can be determined practically quantitatively by this reaction. By the introduction of the bromo-benzoyl radical into the molecule of methyl alcohol, a compound of low molecular weight ($\text{CH}_3\text{O} = 32$) is converted into one of high molecular weight ($\text{C}_6\text{H}_4\text{Br.COOCH}_3 = 215$). The theoretical yield of ester from 1 gram of methyl alcohol is 6.75 grams. Therefore the delicacy of the test is very great. Even quantities of only 0.05–0.1 gram of methyl alcohol in dilutions of 1:200 to 1:400 still give weighable amounts of the methyl ester of p-bromo-benzoic acid. By employing the following

¹W. Autenrieth: Detection of Methyl Alcohol as the Methyl Ester of p-Bromo-Benzoic Acid. Arch. d. Pharm. 258 (1920), 1.

procedure, 55-60 per cent of the theoretical quantity of ester may be obtained. Add to the given aqueous solution in a glass-stoppered flask 10-15 cc of sodium hydroxide solution, heat in a water-bath until the contents of the flask are at 40-45°, then add 2-3 grams of ground p-bromo-benzoylchloride, and shake vigorously until cold. The reaction of the mixture should remain alkaline. Otherwise add 2-3 cc more of sodium hydroxide solution, shake again and test with litmus paper. Then transfer the mixture, rinsing the flask with 10-15 cc of ether, to a rather small separatory funnel without loss. After shaking with the ether, withdraw this solvent into a dry flask, taking the greatest care not to get in even a drop of the aqueous solution. Extract with a fresh portion of 10 cc of ether and also transfer this ether extract without loss to the flask. Now add to the ether solution 2-3 pieces of anhydrous calcium chloride, allow to stand for

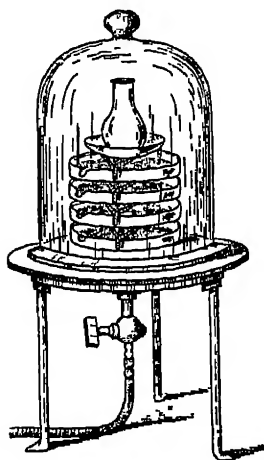


FIG 13—Vacuum bell-desiccator.

some time, pour through a dry filter, rinsing out the flask with a little ether, collect the filtrate in a small, dry, weighed flask, evaporate the ether solution in a vacuum desiccator (Fig 13), and dry to constant weight. In most cases the methyl ester of p-bromo-benzoic acid will be obtained in well crystallized form and have the correct melting-point. For the purpose of recrystallizing the weighed ester, dissolve it in the calculated quantity of cold alcohol, keeping in mind that it is soluble to the extent of 2.3 per cent. Then add an equal volume of water and allow the mixture to stand in ice. Usually the pure ester crystallizes at once and its melting-point may be redetermined.

Example.—The materials taken were: 0.1 gram of methyl alcohol, 20 cc of water, 15 cc of sodium hydroxide solution, and 2.5 grams of p-bromo-benzoyl-chloride. A weight of 0.35 gram of ester was obtained equivalent to 53 per cent of the theory in a methyl alcohol concentration of 1.350.

Detection of Methyl Alcohol in Parts of Cadavers and Urine with p-Bromobenzoylchloride

Since ammonia and ammonium salts as well as volatile phenols, such as phenol and p-cresol, are always formed in the putrefaction of parts of cadavers and also give crystalline compounds with p-bromo-benzoylchloride in presence of sodium hydroxide solution, that is, p-bromo-benzamide, $C_6H_4BrCO.NH_2$, and the phenyl ester of p-bromo-benzoic acid, $C_6H_4BrCOOC_6H_5$, as well as the cresyl ester, parts of cadavers and urine should first be strongly acidified and then subjected to distillation to prevent ammonia from passing over. If a portion of the distillate gives a red color when warmed with Millon's reagent, phenols are present. To hold back the latter, render the rest of the distillate strongly alkaline with sodium hydroxide solution and distil once more. The distillate now obtained may be used for the detection of methyl alcohol with p-bromo-benzoylchloride and sodium hydroxide solution.

Identification of Methyl Ester of p-Bromo-benzoic Acid—1 By its anise-like odor, 2 By its sharp melting-point ($78-79^{\circ}$), 3 By the determination of bromine according to the Carius or the fusion method (37.21 per cent), 4 By converting it by means of strong aqueous or alcoholic ammonia into p-bromobenzamide, crystallizing in leaflets and having the melting-point 188°

Notes—The bell-desiccator¹ for vacuum-drying shown above (Fig. 13) has rendered excellent service in evaporating ether and chloroform solutions, as well as in drying the methyl ester of p-bromo-benzoic acid and many other substances *in vacuo* to constant weight. In using concentrated sulphuric acid as a drying-agent only enough acid to cover the bottom of the flat dishes should be taken.

Formic Acid

Formic acid, H COOH , hardly ever comes up as a poison. If this acid in concentrated form gets upon the skin it may produce blisters and even cause necrosis. Injected under the skin, even small doses of formic acid give rise to pain, hyperemia and inflammation (R. Kobert). Formic acid possesses special toxicological interest for the reason that it occurs in the urine in larger quantity during methyl alcohol poisoning. According to v. Jaksch and v. Rokitsansky, every normal human urine contains small quantities of volatile fatty acids, that is principally acetic acid together with a little formic acid and probably also butyric acid. Salkowski² found an increased amount of formic acid in leukemia. According to Autenrieth,³ formic acid may be regarded as a normal constituent of human urine, for he detected at least traces of this acid in all urines examined. The fluctuation in the percentage of formic acid in the urine of the same individual during a period of 4 months was not very great. Upon the average a person eliminates in the urine 0.251 gram of formic acid in the course of 24 hours. After a dose of methyl alcohol the formic acid-content of the urine shows a considerable increase. A man, who had taken 80 grams of pure methyl alcohol highly diluted during a period of 8 days, eliminated in this time 5.3 grams more formic acid than he did normally. The formic acid-content of the urine is also strongly increased after taking a salt of formic acid. After a dose of 20 grams of sodium formate, distributed over 2 days, the formic acid output was increased about 2.4 grams, that is, 18 per cent of the formic acid taken escaped oxidation in the body of the individual (W. Autenrieth). In toxicologico-chemical investigations the mere qualitative detection of formic acid in urine is not sufficient basis for a final decision in a case of suspected methyl alcohol poisoning. A quantitative determination of formic acid in the urine of the person poisoned is absolutely essential before an opinion not open to question can be rendered.

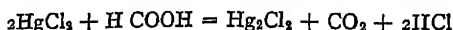
¹ These vacuum desiccators can be procured from the firm of Dr. Bender and Hobein in Munich, Germany.

² E. Salkowski: Formation of Volatile Fatty Acids in Ammoniacal Fermentation of Urine. *Zeitschr. f. physiol. Chem.* 13 (1889), 264.

³ W. Autenrieth: Formic Acid Content of Urine Normally and after Administration of Different Substances. *Münch. med. Wochenschr.* 1919, No. 31, page 862.

Quantitative Estimation of Formic Acid

Formic acid is slightly volatile with steam and actually passes over with more difficulty than does acetic acid. Ordinarily it is determined quantitatively by the method of Scala-Lieben.¹ This is based upon the reduction of mercuric chloride by formic acid to mercurous chloride which is finally weighed.



This method requires rather long warming with a considerable excess of mercuric chloride. Since various aldehydes volatile with steam, such as formaldehyde, acetaldehyde and furfural, in addition to formic acid will also cause reduction of mercuric chloride with heat, separation of formic acid from aldehydes present becomes necessary. The best procedure for this purpose is to add calcium carbonate to the distillate and evaporate.

Procedure—Add 30 cc of 25 per cent phosphoric acid and a few pieces of pumice-stone to 300 cc of the given urine in a 750 cc distilling flask. Use a still-head and conduct distillation at a very vigorous boil. Many urines froth badly when they begin to boil and for this reason only a small flame should be used during the first 10 minutes of distillation. This difficulty, however, is soon over and then the liquid boils quietly. Collect the distillate in a graduate. When about 300 cc, but no more, have passed over and about 30 cc remain in the flask, stop distillation and add 30 cc of water to the flask. Again distil off the same volume and repeat until the distillate fails to react acid with litmus paper. This point is usually reached when 1500–2000 cc. of distillate have passed over. Stir an excess of calcium carbonate with water and add to the distillate. Bring at once to a boil, then evaporate in a flat dish upon the water-bath to 20–30 cc and filter. Rinse dish and filter several times with a little hot water. Transfer the entire filtrate to an Erlenmeyer flask and add 50–60 cc of cold saturated mercuric chloride solution. Shake frequently and heat for 5–6 hours in the water-bath under a reflux. Then add according to the amount of precipitate 2–3 cc of 20 per cent hydrochloric acid, shake and warm 10 minutes longer in the water-bath. Collect mercurous chloride upon a filter dried at 100–110°, or in a Gooch crucible, wash with water until free from chlorine, then with alcohol followed by ether, dry for 1 hour at 100–110° and weigh. To calculate the quantity of formic acid, multiply the weight of mercurous chloride precipitate by 0.0977.

Fincke² adds 3–5 grams of crystallized sodium acetate to the neutral or faintly acid solution of formic acid, the volume of which in the case of rather small quantities should be 50–100 cc and for larger quantities, over 50 milligrams of formic acid, 100–300 cc, and a quantity of mercuric chloride at least 15 times that of the formic acid. The entire mixture is put into an Erlenmeyer flask, provided with a rubber stopper and a glass tube 30–40 cm long for a reflux, and

¹ A. Scala. Quantitative Estimation of Formic Acid in Presence of Acetic Acid and Butyric Acid. *Gazz. chim. Italian* 20 (1860), 393; A. Lieben: Estimation of Formic Acid. *Monatsh. f. Chem.* 14 (1893), 747.

² H. Fincke. Contribution to the Estimation of Formic Acid in Foods. *Zeitschr. f. Nahrungs- u. Genussm.* 22 (1911), 88.

heated for 2 hours in an actively boiling water-bath Mercurous chloride is filtered hot and otherwise treated as described above

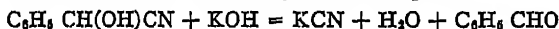
Notes.—Use of a caustic alkaline solution instead of calcium carbonate for the neutralization of formic acid is not permissible, because aldehydes are held back in the form of aldehyde resins and formaldehyde present may possibly be decomposed to methyl alcohol and formic acid

Estimation of Formic Acid in Presence of Salicylic Acid

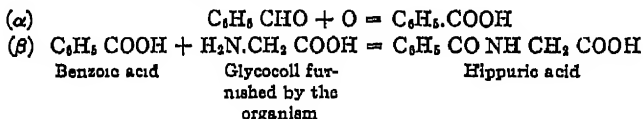
If the solution of a salicylate is heated with mercuric chloride solution in presence of sodium acetate, as in Fincke's method of estimating formic acid, turbidity appears and gradual separation of a compound of mercury and salicylic acid takes place. This separation may be prevented by addition of sodium chloride, so that formic acid can be estimated by means of mercuric chloride in presence of salicylic acid. Of course too large quantities of sodium chloride affect the accuracy of the estimation of formic acid

BITTER ALMOND WATER AND OIL OF BITTER ALMONDS

Bitter almond water (*Aqua Amygdalae Amarae* of the Pharmacopoeia) contains hydrocyanic acid. Only a small portion of this acid, however, is free so that it can be precipitated by silver nitrate solution. The greater part is combined as the cyanohydrin of benzaldehyde which does not react with silver nitrate. But potassium hydroxide solution will decompose this compound



Pure benzaldehyde, also called hydrocyanic acid-free oil of bitter almonds, is not poisonous. It is oxidized to benzoic acid in the body and eliminated in the urine, partly as that acid and partly as hippuric acid, after conjugation with glyccoll (amino-acetic acid).



Ordinary commercial oil of bitter almonds contains hydrocyanic acid and is poisonous in proportion to the quantity of this acid present. To test for hydrocyanic acid, shake a few cc. of oil of bitter almonds with 10 cc. of potassium hydroxide solution, separate the latter from the oil, and make the Prussian blue test.

When oil of bitter almonds is mixed with other material, distil with steam from a solution acidified with tartaric, or dilute sulphuric acid, and test the first part of the distillate for hydrocyanic acid. If benzaldehyde is present, the distillate at the same time will be milky and have the characteristic odor of that compound. Distil until the drops of water are perfectly clear. Benzaldehyde may be detected with certainty, and at the same time distinguished from the highly poisonous nitro-benzene which has a somewhat similar odor, by adding a few drops of potassium hydroxide solution to the milky distillate, to combine with any hydrocyanic acid, and extracting with ether. The ether upon evaporation

will deposit benzaldehyde as oil-drops which can be positively identified by conversion into benzoic acid. Heat the oil-drops for a few minutes in a small flask provided with a reflux with about 10 cc of potassium dichromate solution and a little dilute sulphuric acid. Cool, extract with ether, and evaporate the ether solution in a glass dish. When the material contains benzaldehyde, this residue will consist of benzoic acid. This substance may be further identified by its melting-point ($120-121^{\circ}$), its property of subliming, and the test with ferric chloride solution ¹.

To test for nitrobenzene in a distillate that has an odor like that of bitter almonds, shake with tin and hydrochloric acid (see page 70) and then test for aniline.

With regard to the "*Estimation of minute quantities of hydrocyanic acid colorimetrically*" and the "*Examination of bitter almond water*," see Chapter V, pages 540 and 543 of this book.

CARBON DISULPHIDE

Carbon disulphide, CS_2 , is a colorless liquid having a characteristic odor and a high index of refraction. As the following table shows, there is some difference of opinion with regard to the solubility of carbon disulphide in water:

Temperature	1000 cc of water dissolve
$13-14^{\circ}$	2.03 parts (Page)
$15-16^{\circ}$	1.81 parts (Chancel, Parmentier)
$15-16^{\circ}$	2.0-3.0 parts (Ckundi)
$15-16^{\circ}$	3.5-4.52 parts (Péligot)

Carbon disulphide is miscible in all proportions with absolute alcohol, ether, ethereal and fatty oils.

Toxic Action.—Carbon disulphide administered internally has a very poisonous action upon the blood, causing especially decomposition of red blood-corpuscles. Even inhalation of carbon disulphide vapor frequently occasions severe poisoning. Carbon disulphide was formerly considered a typical producer of methaemoglobin but recent investigations have not confirmed this opinion. It has a very injurious action upon the red blood-corpuscles, causing haemolysis. R. Kobert (Intoxikationen) states that its power of dissolving lipoids is responsible for its injurious action upon the blood and the central nervous system. Harmsen² has recently come to practically the same conclusion. He considers carbon

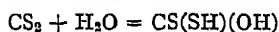
¹ Dissolve the residue in a small quantity of water and neutralize benzoic acid by heating the solution to boiling with excess of calcium carbonate. Filter and add a few drops of ferric chloride solution. If benzoic acid is present, a flesh-colored precipitate of basic ferric benzoate will appear. Tr.

² E. Harmsen. Carbon Disulphide Poisoning in Factories. *Vierteljahrsschr. f. gerichtl. Med. usw.* 30 (1905), 422.

disulphide a powerful blood-poison, because it decreases the number of red blood-corpuscles and the quantity of haemoglobin and brings about a leucocytosis ¹

Detection of Carbon Disulphide

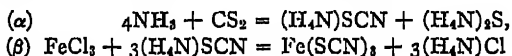
Carbon disulphide distils very slowly with steam. Consequently the second or third fraction of the distillate should be used in testing for this substance. If 40 cc are distilled from 100 cc of water, containing 2 drops of carbon disulphide, the following 10 cc will give a distinct test. If the quantity of carbon disulphide is small, it will remain in solution. Such a solution does not have a strong odor. It is not impossible that the aqueous solution of carbon disulphide contains dithio-carbonic acid.



Carbon disulphide may be recognized in the distillate by the following tests:

1. Lead Acetate Test.—Add a few drops of lead acetate solution to the liquid containing carbon disulphide. It will cause neither a precipitate (distinction between CS_2 and H_2S) nor a color. Add excess of potassium hydroxide solution and boil. A black precipitate (PbS) will appear. This is a very delicate test.

2. Sulphocyanate Test.—Heat an aqueous solution of carbon disulphide for a few minutes with concentrated ammonium hydroxide and alcohol. Ammonium sulphocyanate ($\text{H}_4\text{N}(\text{SCN})$) is formed together with ammonium sulphide. Concentrate this solution upon the water-bath to about 1 cc and acidify with dilute hydrochloric acid. Add a drop of ferric chloride solution and a deep red color will appear. This test will show even traces of carbon disulphide, for example, 0.05 gram in 1 cc. of water.



3. Xanthogenate Test.—Shake a few cc. of a distillate containing carbon disulphide for several minutes with about 3 times its volume of saturated solution of potassium hydroxide in absolute alcohol. Faintly acidify the solution with acetic acid and add 1-2 drops of copper sulphate solution. If carbon disulphide is present, a brownish black precipitate of cupric xanthogenate, will appear. This will

¹ Leucocytosis is a temporary increase in the number of white blood-corpuscles (leucocytes) as compared with the number of red blood-corpuscles. Normally there are about 350 red to 1 white blood-corpuscle, whereas in leucocytosis the proportion is 20:1.

soon change to a yellow, flocculent precipitate of cuprous xanthogenate, $\text{CS}(\text{SCu})(\text{OC}_2\text{H}_5)$

Quantitative Estimation of Carbon Disulphide in Air

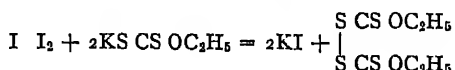
Inhalation of air containing carbon disulphide has frequently given rise to chronic poisoning. Individuals thus affected have usually been workmen in rubber factories. Consequently experiments have been made to determine the maximum quantity of carbon disulphide air may contain without injury to health. The results of these investigations may be summarized as follows.

No.	CS_2 in mgrs per liter of air	Result
1.	0.5-0.8	No injurious effect
2.	1.3	Slight uneasiness after several hours
3.	3.4	Uneasiness in 30 minutes
4.	6.0	Uneasiness in 20 minutes
5.	10.0	Paralysis attended by after- effects lasting several days

The exact danger limit for persons obliged to live for weeks at a time in an atmosphere containing carbon disulphide should be placed below 3 milligrams in a liter of air. Air in factories, where operatives work in presence of carbon disulphide vapor, should never exceed this limit. Since experiments have shown that 93-96 per cent. of the carbon disulphide breathed was exhaled unchanged, an exceedingly small quantity is capable of producing toxic symptoms.

Procedure.—To determine quantitatively the amount of carbon disulphide in the air, place a saturated alcoholic solution of potassium hydroxide in a Pélignot absorption-tube and draw through this solution 10-20 liters of air containing carbon disulphide vapor. A quantitative formation of potassium xanthogenate, $\text{CS}(\text{SK})(\text{OC}_2\text{H}_5)$, will take place. At the end of the experiment dilute the contents of the receiver with 96 per cent. alcohol and bring the volume to 50 cc. Measure an aliquot portion of this solution and dilute with water. Faintly acidify the solution with acetic acid and remove excess of acid with acid sodium carbonate. Add freshly prepared starch solution and 0.1 N iodine solution until there is a permanent blue color.

Explanation—Iodine converts potassium xanthogenate according to equation (I) into ethylxanthogen disulphide



Rupp and Krauss¹ think the action of iodine upon potassium xanthogenate is expressed by equation (II)



Both equations require the same quantity of iodine, that is, 2 atoms for 2 molecules of xanthogenate. A difference therefore in the mechanism of the reaction has no influence upon the combining relations of the iodine, and the method is applicable to the quantitative determination of xanthogenate

1000 cc of 0.1 N-iodine solution, containing 0.1 gram-atom of iodine, correspond to 0.1 gram-molecule of $\text{CS}_2 = 7.6$ grams.

Preservation of Cadaveric Material for Toxicological Examination

Various liquids used to preserve cadaveric material for later toxicological examination, such as sulphur dioxide, chlorine water, calcium chloride, other metallic salts, and alcohol itself, possess certain disadvantages and often fail to meet the requirements. A satisfactory preserving solution should make it impossible for histological or chemical changes to take place in tissues. It should not introduce any foreign substance into the material to be preserved either itself or impurities that it may contain. According to Mameli,² liquid air fulfils these requirements. Every putrefactive process, as well as every chemical action, is checked by its low temperature (-190°). It prevents loss of poisons that are easily volatile or alterable, such as carbon monoxide and hydrocyanic acid. Yellow phosphorus is not oxidized when kept for some time in liquid air. For this reason it was found possible to get a positive Mitscherlich test in the case of a rabbit to which phosphorus had been given. The stomach and contents were preserved for 40 hours in liquid air. An additional advantage of this method is that cadaveric material preserved in liquid air becomes so brittle that it can be ground in a mortar.

Grigorjew³ has recommended formalin as a preserving solution for cadaveric material and Barth⁴ has carried on extensive experiments upon its use for this purpose. The material used for examination was fresh beef liver finely chopped. It was covered with the preserving fluid and allowed to stand at room temperature for varying lengths of time in glass jars covered with parchment paper and kept where it was light. The poisons selected were the alkaloids, morphine, strychnine, atropine and veratrine, and arsenic, phosphorus, potassium cyanide

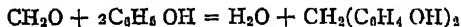
¹ E. Rupp and L. Krauss: Iodimetric Estimation of Copper as Cuprous Xanthogenate. *Ber. d. Deutsch. chem. Ges.* 35 (1902), 4157.

² E. Mameli: Use of Liquid Air in Toxicology. *Boll. della Società Medico-chirurg. di Pavia* 1910.

³ A. Grigorjew: Preservation of Parts of Cadavers. *Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw.* 29, 79.

⁴ Barth: *Pharm. Journ.* 44 (1905), 51.

and phenol. At the same time parallel experiments were made with distilled water, alcohol, and glycerol. In detecting alkaloids, the Stas-Otto process was used. From the results obtained in these experiments, Barth drew the following conclusions. Formalin is much to be preferred to alcohol as a preservative because material kept in it gives purer extracts. The free alkaloids pass in purer condition into the ether and chloroform extracts and do not have to be converted into salts for purposes of purification. When formalin was used as a preservative, 0.02 gram of alkaloid could be detected even after 12 months, whereas when alcohol was used no test for 0.02 gram of atropine was given after the same period of time. Phosphorus is very well preserved by formalin. Potassium cyanide and carbolic acid, however, did not give as favorable results. No test for hydrocyanic acid was given in the case of 0.02 gram of potassium cyanide in 5 and 10 per cent formalin solution, although the material was well-preserved.¹ But a test for hydrocyanic acid was obtained where liver, containing 0.2 gram of potassium cyanide, was preserved in 1 per cent formalin solution, because the quantity of formalin was not sufficient to combine chemically with the entire quantity of potassium cyanide present. The outcome of the experiments with phenol was the same. Phenol could not be detected with ferric chloride, when less than 0.5 per cent was in 10 per cent formalin solution, not even immediately after mixing the two solutions. These two substances must react as shown in the following equation:



On the other hand, ferric chloride gave a test when larger quantities of phenol were used.

Although formalin possesses many advantages over alcohol, it is not always to be recommended as a preservative of cadaveric material that is to be examined for poisons. Under no circumstances should formalin be used, when it is a matter of using the material for the detection of hydrocyanic acid, carbolic acid or other volatile phenols.

¹ The addition-product, $\text{CH}_2(\text{OK})(\text{CN})$, may be formed by the interaction of formaldehyde and potassium cyanide.

CHAPTER II

NON-VOLATILE POISONS

Organic Substances Non-volatile with Steam from Acid Solution but Extracted by Acidulated Alcohol¹

Put a portion of finely chopped material into a large flask and thoroughly mix with 2-3 times as much absolute alcohol ² Add enough tartaric acid solution to give the mixture an acid reaction after shaking Laboratory experiments usually require 20-30 drops of 10 per cent. tartaric acid solution Avoid a large excess of tartaric acid, since it may act as an objectionable impurity in the ether extract, owing to its solubility in that solvent Connect the flask with a glass tube (80-100 cm long) serving as a reflux. Frequently shake and heat 10-15 minutes upon the water-bath In the extraction of a large quantity of material from a cadaver, connect the flask with an upright Liebig condenser used as a reflux (Fig 14) Cool the flask contents and filter to remove fat as completely as possible. Wash the residue with alcohol Evaporate the filtrate, which should have an acid reaction,³ to a thin syrup in a glass dish upon the water-bath Thoroughly mix this residue with 100 cc of cold water. Usually this causes an abundant separation of fat and resinous matter, especially when parts of a cadaver are examined Filter through a moistened creased paper and evaporate the filtrate to dryness, or to a syrup, upon the water-bath Thoroughly mix this residue with absolute alcohol As a result of this treatment, a

¹ Artificial mixtures for laboratory practice should be examined according to the method outlined above They may consist of dog-biscuit, meat, comminuted organs (liver, kidneys, spleen, etc), sausage meat, or other materials mixed with any of the poisons of this group The same systematic procedure should be followed in examining soup, milk, vegetables, and potatoes for these poisons

² Commercial alcohol usually contains basic compounds, the presence of which is objectionable They should be removed by adding tartaric acid to the alcohol and distilling Alcohol should not be used in toxicological analysis, unless an actual test has shown it to be free from such impurities. Tr

³ If this filtrate is not acid (litmus paper test), it should be acidified with a few drops of tartaric acid solution

whitish substance, more or less viscous or slimy, usually remains undissolved. This residue, consisting chiefly of protein substances (albumin, albumoses and peptones), dextrin-like compounds, and in part also of inorganic salts, frequently becomes granular upon standing. Tartrates of the alkaloids and other organic poisons are dissolved. The larger the quantity of absolute alcohol used, the more

complete is the precipitation of those substances that interfere more or less with the detection of organic poisons. Again evaporate the filtered alcoholic solution upon the water-bath and dissolve the residue in about 50 cc of water. If the solution is not perfectly clear, filter through a moistened paper.

The result of this procedure is a solution containing alkaloidal tartrates and other organic substances belonging to this group. The solution should have an acid reaction and be practically free from protein substances, fat, resinous bodies, and coloring-matter. If the solution fulfils these requirements, it is ready to be examined for organic poisons according to the "Stas-Otto" method. The utmost care should be taken in preparing this solution, for definite conclusions cannot be drawn from the uncertain tests given by impure material.

This aqueous acid solution should be examined according to the Stas-Otto process in the following manner:

A. Thoroughly extract the acid solution several times with ether. This solvent will dissolve free acids and acid compounds, such as picric acid, salicylic acid, veronal; neutral substances, such as acetanilide and phenacetine; and very weak bases, such as antipyrine, caffeine, colchicin and narcotine. But only traces of the last-mentioned substances pass from the tartaric acid solution into the ether.

B The acid aqueous solution remaining after extraction with ether should be made strongly alkaline with sodium hydroxide solution and again extracted with ether. Sodium hydroxide sets

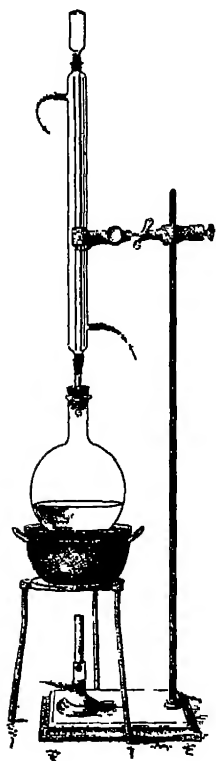


FIG 14—Liebig condenser as reflux.

free basic substances, that is, most of the alkaloids with the exception of phenolic bases, such as morphine and apomorphine which remain in the aqueous solution as sodium compounds. Ether dissolves these liberated bases.

C. The aqueous solution alkaline with sodium hydroxide must be changed to one alkaline with ammonia. This can be brought about by adding ammonium chloride. Excess of sodium hydroxide is combined and any sodium compounds of phenolic bases that may be present are at the same time decomposed¹. Some analysts, however, recommend neutralizing the sodium hydroxide solution with dilute hydrochloric acid and then making alkaline with ammonia. This aqueous ammoniacal solution should then be extracted as follows.

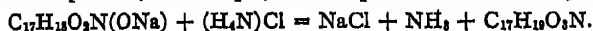
(α) Ether² will extract apomorphine and traces of morphine

(β) Hot chloroform, or according to the Stas-Otto process pure amyl alcohol, will extract morphine and narceine from the aqueous solution. This chloroform extract may also contain colchicin, caffeine and antipyrine, as well as those substances of *A* and *B* that are only slightly soluble in ether. Kippenberger acidifies the alkaline aqueous solution, after extraction with ether, with dilute hydrochloric acid. He then renders this solution alkaline with sodium bicarbonate instead of with ammonia and extracts morphine, narceine, and the other substances that belong here, with a hot mixture consisting of 90 volumes of chloroform and 10 volumes of absolute alcohol. The author highly recommends this hot alcohol-chloroform mixture for the extraction of morphine.

Examination of a Powdered Mixture Containing Cane-Sugar or Milk-Sugar

When the material is a powder mixed with cane-sugar or milk-sugar, it is usually possible, after the aqueous solution has been acidified with tartaric acid, to extract direct. The acid solution is

¹ Sodium morphinate, for example, is decomposed in the following manner.



² This ether extraction (α) of the ammoniacal solution is not necessary, unless the ether extracts of the acid aqueous solution, or of this solution after it has been made alkaline with sodium hydroxide, have a red or violet color due to colored decomposition products of apomorphine, that is, when these colors point to the presence of apomorphine.

filtered hot and examined for poisons by the Stas-Otto method according to the procedure outlined above under *A*, *B* and *C*

Examination of Beer, Wine and Infusions of Cocoa, Coffee and Tea

In the examination of such material, which is frequently brought to the forensic chemist to be tested for poison, the process outlined above may be considerably abridged. Acidify the material with aqueous tartaric acid solution, if necessary, and evaporate in a glass dish upon the water-bath. Treat the residue thoroughly with absolute alcohol and filter. Evaporate the filtrate upon the water-bath and dissolve the residue in tepid water. Filter this solution, if necessary, and then examine according to the Stas-Otto process.

A Preparation and Examination of Ether Extract of Aqueous Tartaric Acid Solution

Thoroughly extract the acid aqueous solution (see process of preparation described above) 2-3 times with ether, using each time about the same quantity of solvent. Employ a separating funnel for this purpose (Fig 15). Pour the combined ether extracts into a dry flask loosely stoppered. If the solution stands for 1-2 hours at rest, a few drops of water usually settle out. Decant the ether solution and pour through a dry filter. Slowly evaporate this solution in a small glass dish upon a water-bath previously heated slightly above 35°. **Do not have gas burning during this operation!** Examine the residue as described below. An excellent method of evaporating ether consists in setting a small glass dish (8-10 cm. in diameter) upon a hot water-bath and dropping the filtered ether extract into it as fast as the solvent evaporates. Thus a large quantity of extract may be evaporated in a small dish. The advantage of this method is the ease with which the residue can be removed for the various tests. The residue is usually quite small and it is not advisable to have it distributed over too large a surface. Larger quantities of ether solution are distilled in a small dry flask upon the water-bath until about 10 cc. remain. The latter may then be evaporated upon a small watch-glass.

Examine the residue from the ether extract for the following substances:

Picrotoxin Picric Acid Acetanilide Veronal Caffeine
Colchicin Salicylic Acid Phenacetine Antipyrine Cantharidin

The feebly basic substances, antipyrine and caffeine, appear only in small quantity in the ether extract of the tartaric acid solution. By far the greater part of these two substances is extracted by ether or chloroform from the aqueous solution after it has been rendered alkaline with sodium hydroxide or ammonia. The very feebly basic colchicin, which is only slightly soluble in ether, also appears

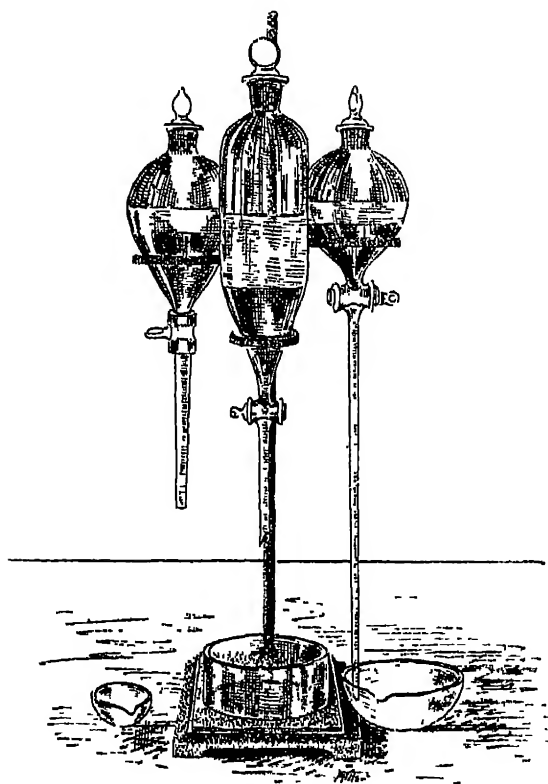


FIG 15.—Separating funnels and glass crystallizing dishes

mainly in the chloroform extract of the aqueous solution after it has been rendered alkaline with ammonia.

Evaporation of the ether extract, even in the absence of members of the group, usually leaves a more or less viscous residue, containing tartaric and lactic acid as well as fatty, resinous and colored substances. This is particularly the case in analyses of cadaveric

material. Moreover, ether extracts certain metallic salts from aqueous solutions, for example, mercuric cyanide¹ and chloride.

First, note the general appearance and taste of the residue. Then examine it with a microscope. Very definite conclusions as to the presence or absence of certain substances can frequently be drawn. A very bitter residue should be examined especially for picrotoxin and colchicin. If there is a pronounced yellow color, the examination should include picric acid also. Veronal is colorless and has a pleasantly bitter taste. The same is true of caffeine. A tasteless or only faintly bitter residue probably does not contain these substances and should be examined for acetanilide, antipyrine, phenacetine and salicylic acid.

The residue from evaporation of the ether extract may contain the following substances.

Picrotoxin.—Usually a thick syrup that gradually solidifies and becomes crystalline. It has an intensely bitter taste.

Colchicin.—Yellowish, amorphous residue that does not become crystalline. Tastes intensely bitter. Dissolves in water with a yellow color.

Picric Acid.—Usually appears as a syrup that gradually solidifies and becomes crystalline. Tastes very bitter. Residue intensely yellow, giving yellow aqueous solutions.

Acetanilide.—Leaflets or flat needles. Has a faint burning taste but is not bitter.

Phenacetine.—Inodorous and tasteless leaflets and small needles.

Antipyrine.—Residue a syrup that rarely crystallizes. Tastes mildly bitter. Very easily soluble in water.

Salicylic Acid.—Crystallizes in small needles. Has a harsh and at the same time sweet, acidulous taste.

Veronal.—Leaflets and needles having a bitter taste.

Caffeine.—Residue composed of small needles frequently in radiating clusters. Tastes mildly bitter.

¹ To some extent ether will extract mercuric cyanide from a tartaric acid solution that is not too dilute. For example, it will remove appreciable quantities from 100 cc. of 0.1 per cent. mercuric cyanide solution but the extraction will not be complete. The solution after five extractions will still give a distinct test for mercury. Ether will not remove even a trace of mercuric cyanide from 0.01 per cent. solution. To test for cyanide, add ammonium sulphide solution to the ether residue. This will precipitate mercuric sulphide and the filtrate will contain ammonium sulphocyanate (see hydrocyanic acid, page 30).

The residue from the ether extract evaporated upon a watch-glass should not be heated upon the water-bath longer than necessary, for various substances becomes viscous and salicylic acid, if present, may volatilize. Remove the watch-glass as soon as the odor of ether is gone. Scrape together the cold residue by means of a platinum or nickel spatula, or clean knife-blade, dividing it into several portions and testing for the poisons that may appear.

The author has frequently found the apparatus shown in Fig. 16 useful in distilling or evaporating larger quantities of liquid *in vacuo*. The vacuum apparatus consists of a porcelain basin, suspended in a water-bath and tightly connected by a broad, well-ground edge with a vacuum-bell. A tight connection

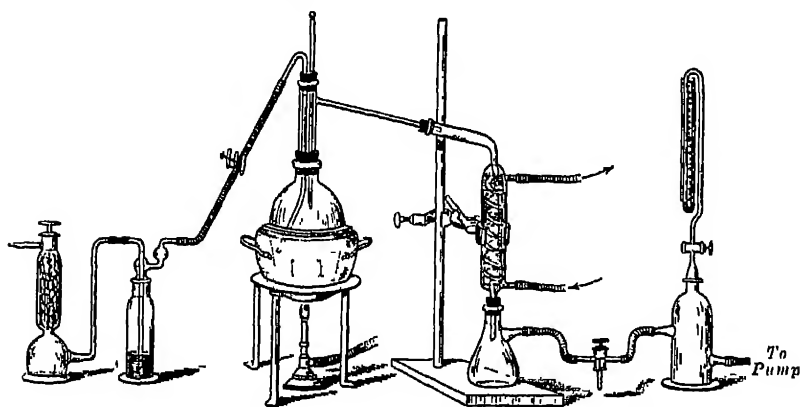


FIG. 16—Apparatus for distillation or evaporation in *vacuo*

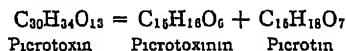
can be secured by using a rubber ring. But the latter is unnecessary if the porcelain basin and vacuum-bell fit well. The liquid to be distilled or evaporated may be placed in the porcelain basin itself, or in any containing-vessel, such as a porcelain dish, watch-glass, beaker, etc., set in the basin.

PICROTOXIN

Picrotoxin, $C_{30}H_{54}O_{18}$, the poisonous principle of *Cocculus indicus*, the fruit of *Menispermum Cocculus*, is present to the extent of 5 per cent. Owing to its stupefying action upon fish, it is employed in catching them, and because of its very bitter taste is said to have found use as a substitute for lupulin.

Properties.—Picrotoxin crystallizes from hot water in long, colorless needles melting at $199-200^{\circ}$. It dissolves with difficulty in cold water but considerably more easily in hot water or alcohol. It is only slightly soluble in ether but freely soluble in chloroform, amyl alcohol and glacial acetic acid. Its alcoholic solu-

tion is neutral and laevo-rotatory. Picrotoxin has a very bitter taste. It is soluble in caustic alkalies and aqueous ammonia, forming unstable, salt-like compounds that do not crystallize. Picrotoxin has therefore the character of a weak acid. After prolonged boiling with considerable benzene, it is decomposed into picrotoxinn and picrotin. The former passes into solution but picrotin is almost completely insoluble.



Chloroform brings about this cleavage even more easily.

On the other hand, if picrotoxinn and picrotin in molecular proportions are dissolved in hot water, picrotoxin crystallizes out as the solution cools. Treated with bromine direct or dissolved in water or ether, picrotoxin is first split into picrotoxinn and picrotin. The former is immediately converted into monobromo-picrotoxinn, $\text{C}_{16}\text{H}_{15}\text{BrO}_6$, but picrotin remains almost unchanged. Monobromo-picrotoxinn is soluble with difficulty in water but is reduced by zinc dust and acetic acid to picrotoxinn. Picrotoxinn has a very poisonous action, whereas picrotin is almost non-toxic.

Meyer and Bruger¹ regard picrotoxin as a complex of the two compounds, picrotin and picrotoxinn, crystallizing together in definite but not molecular proportion, and not as a molecularly constituted chemical compound.

Toxic Action.—Picrotoxin is a powerful convulsive poison, standing in its action between cicutoxin and strychnine. Its absorption takes place easily. A few minutes after the poison has been taken, the first symptoms of intoxication appear (emesis, diarrhoea, dizziness, enlargement of the pupils, epileptic or tetanic convulsions, accompanied by peculiar cries, flow of saliva, sweating and delirium). The poison in part is eliminated unchanged in the urine which should first be tested in case of poisoning by picrotoxin. Kobert has described a case of poisoning, the course of which was extremely violent. A man, who had taken tincture of *Cocculus indicus* in small doses for a week and then a larger dose at one time, was taken with dizziness, nausea and very soon with a convulsion that threw him to the floor. Tonic and clonic convulsions followed with loss of consciousness and 30 minutes after the dose was taken death ensued. The lethal dose is small. In one case death resulted from 2.4 grams of the seed.

Detection of Picrotoxin

Picrotoxin, picrotoxinn and picrotin act as reducing agents.

1. Fehling's Test.—Dissolve picrotoxin in a small test-tube, using 10–20 drops of very dilute sodium hydroxide solution. Add a few drops of Fehling's solution² and warm but do not shake. A red or yellowish red precipitate gradually forms and settles to the bottom. If the ether residue, not too little of which should be taken, fails to

¹ R. J. Meyer and P. Bruger: Contribution to the Knowledge of Picrotoxin. *Ber. d. Deutsch. chem. Ges.* 30 (1898), 2958.

² Fehling's solution heated by itself should not give a precipitate of cuprous oxide.

give a clear solution in very dilute sodium hydroxide solution, filter through moistened paper and examine the filtrate with Fehling's solution.

2. Ammoniacal Silver Test.—Warm picrotoxin with aqueous silver nitrate solution containing a slight excess of ammonium hydroxide solution. The reducing action of picrotoxin will produce a black precipitate of metallic silver, or a dark brown color when only traces are present.

3. Oxidation Test.—Picrotoxin, treated with a little concentrated sulphuric acid in a porcelain dish, first becomes orange-red and then dissolves when stirred forming a reddish yellow solution. A drop of potassium dichromate solution will produce a red-brown color around the margin of the drop. If the two liquids are thoroughly mixed, an immediate dirty brown color appears, passing into green on long standing.

A green color alone is without significance, since many organic substances capable of reducing chromic acid to chromic oxide produce the same result.

4. Melzer's Test.¹—Put some picrotoxin upon a watch-glass and add 1–2 drops of a mixture of benzaldehyde and absolute alcohol. Careful addition of a drop of concentrated sulphuric acid will produce a distinct red color. If the watch-glass is tilted back and forth, red streaks will run from the substance through the liquid.

Use a freshly prepared 20 per cent. solution of benzaldehyde in absolute alcohol. Benzaldehyde alone gives a yellowish brown color with concentrated sulphuric acid. Alcohol is added as a diluent to diminish this color as much as possible. Under these conditions the solution has a light yellow color, and the dark red tint caused by picrotoxin is very clearly defined. This red color is unstable and, beginning at the margin, gradually fades into a pale pink or violet. Kreis² has found that cholesterol and phytosterol³ give similar colors with Melzer's reagent. Veratrine gives a red color like that with sulphuric acid alone. Morphine gives fine red to yellow-red streaks or colors.

5. Langley's Test.—Mix picrotoxin with about 3 times the quantity of potassium nitrate and moisten the mixture with the smallest

¹ H. Melzer. Contributions to Forensic Chemistry. *Zeitschr. f. analyt. Chem.* 37 (1898), 351 and 747.

² H. Kreis. Contribution to the Knowledge of Melzer's Picrotoxin Test. *Chemiker-Zeitung* 23 (1899), 21.

³ A substance very similar to cholesterol, and named paracholesterol or phytosterol, is found in the seeds of certain plants (Perkin and Kipping, *Organic Chemistry*, page 574.)

possible quantity of concentrated sulphuric acid. Then add strong sodium hydroxide solution in excess and an intense red color will appear.

6. Physiological Test.—The stupefying, intoxicating action of picrotoxin upon fish may be used for its detection. This test may be made by dissolving the substance to be examined for picrotoxin, depending upon its quantity, in 250–500 cc of spring or tap-water and introducing a small fish weighing about 1 gram. After some time fish reach a condition of great irritation, sometimes turning on their long axis, losing the power of swimming in the normal position, and lying on their sides. One milligram of picrotoxin is said to kill rather small fish in 7 hours and 9.5–10 milligrams in 2.5 hours. The haemolytic action of picrotoxin in physiological salt solution may also be used for its detection; likewise its action in causing convulsions. Picrotoxin is a convulsive poison, unlike strychnine not producing extension of the hind legs. Fühner¹ has made the following observations. If 0.2 mg of picrotoxin is injected into a medium-sized frog, the animal after several hours will no longer rest upon its front legs; it keeps its hind legs mostly in a bent position; and moreover there is tension of the webs. Even after 24 hours there is stiffness and an inclination to take this characteristic “picrotoxin position.” In the case of 0.5 mg the first convulsion occurs after about 45 minutes. The animals may be thrown upon their backs and then into the abdominal position. These convulsions may persist for several hours. With 1 milligram, convulsions that begin to be tetanic in character, appear after about 1 hour. Usually severe convulsions occur later spontaneously, or frequently better after irritation in conjunction with full inflation of the thorax and its sudden evacuation with a sharp cry. These convulsions with and without cry may be repeated for several hours. They gradually become weaker and finally the animal dies completely paralyzed.

Detection of Picrotoxin in Beer and Urine

Start with at least 2 liters of beer. If it has a faint acid reaction, make it neutral with magnesium oxide and evaporate upon the water-bath to a thin syrup. Digest this residue with 5 times its volume of alcohol, evaporate the filtered alcoholic extract, dissolve the residue in hot water, filter through a moistened paper, acidify the filtrate with dilute sulphuric acid, and extract repeatedly with ether, or better with chloroform. Should the residue from ether or chloroform be too impure, dissolve again in hot water, filter, evaporate, and extract with ether or chloroform. To purify picrotoxin further, precipitate colored substances from its aqueous solution with lead acetate, filter and remove lead from the filtrate by hydrogen sulphide. The filtrate from lead sulphide upon evaporation, or extraction with ether or chloroform, will give nearly pure picrotoxin. The very bitter taste of picrotoxin, its strong tendency to crystallize from a little hot water, the chemical reactions given above, and especially the physiological experiment with a small, lively fish, serve for its identification.

The detection of picrotoxin in any other material, such as urine, may be undertaken in the manner described above.

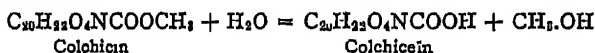
¹ H. Fühner. *Detection and Estimation of Poisons Biologically*, Abderhalden's *Biochemische Arbeitsmethoden*, Vol. V, Part I, Page 46 (1911).

COLCHICIN

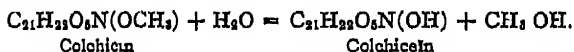
Colchicin, $C_{22}H_{25}NO_6$, an alkaloid occurring in all parts of the meadow saffron, *Colchicum autumnale*, is found most abundantly in the ripe seeds, especially in the hulls (0.3–0.8 per cent.) and in the bulbous buds collected before they bloom (0.2–0.5 per cent.). It occurs in smaller quantity in the fresh flowers (0.01–0.02 per cent.) and in the fresh leaves. According to the statements of other investigators, the fresh flowers contain 0.1–0.6 per cent. of colchicin.

Colchicin is a yellowish, amorphous powder, highly poisonous and very bitter to the taste. It is freely soluble in water, alcohol, and chloroform, less so in ether and benzene, and almost insoluble in petroleum ether. The yellowish solutions of colchicin possess only very weak basic properties. Consequently ether or chloroform, but not benzene or petroleum ether, will extract colchicin from an aqueous, tartaric acid solution. Upon evaporation of the solvent, colchicin will appear as a yellowish, sticky residue resembling a resin or varnish. Extraction of colchicin is more complete, if the aqueous solution is made alkaline with ammonia and extracted with chloroform. Dry colchicin upon exposure to light becomes darker in color.

Constitution of Colchicin.—When boiled with acidulated water, colchicin undergoes cleavage into methyl alcohol and colchiceïn. The latter is a weak acid, containing according to Zeisel a free carboxyl-group, colchicin being the methyl ester of this acid.



Positive proof of the presence of a carboxyl-group in the colchucen molecule has not yet been furnished. According to A. Windaus, the possibility that colchucen is an enol-form must be taken into consideration and that colchicin is the corresponding methyl ether of this enol-form.

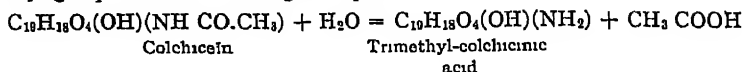


This view of Windaus finds support in the fact that colchicin, but not colchicin, gives a green coloration with ferric chloride. These color-reactions with ferric chloride are characteristic of phenols and enol-forms but not of carboxylic acids.

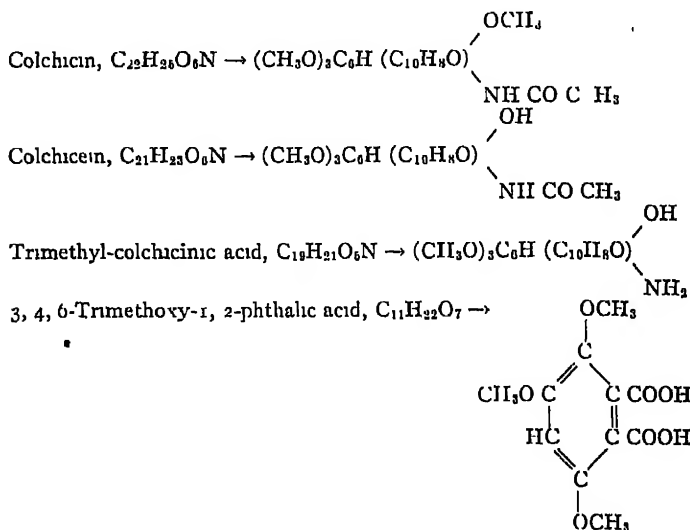
On the other hand, colchicin is formed when colchicein is heated at 100° with sodium methylate and methyl iodide.

Since colchicein upon treatment with hydriodic acid yields three molecules of methyl iodide, colchicein as well as colchicin contains three methoxyl-groups. Heated with strong hydrochloric acid, colchicein loses acetic acid and passes into trimethyl-colchicine acid. The latter is capable of forming salts with

bases and also with acids Zeisel assumes that in the formation of this acid an acetyl-group attached to nitrogen is split off.



According to Zeisel, trimethyl-colchicine acid still contains three methoxyl-groups They are attached to a benzene ring, for by the oxidation of this acid Windaus has obtained 3, 4, 6-trimethoxy-1, 2-phthalic acid For the cleavage-products isolated by Zeisel and Windaus¹ and for colchicin itself the following formulae have been proposed



Toxic Action.—Absorption of colchicin takes place very slowly The symptoms of poisoning are emesis and cholera-like stools, pains in the back, arms and legs, spasms and convulsions, dyspnoea, and disturbances of vision and speech. The body-temperature may drop abnormally, so that the skin feels cold There is intense thirst and nausea, also micturition, and albuminuria may appear Yet the urine may also be normal but always deficient in quantity In consequence of poor absorption of colchicin, symptoms of intoxication usually do not appear for several hours, and its strong cumulative action may be attributed to this cause Colchicin belongs without doubt among the most potent poisons and a single dose of 3 mg has been instrumental in bringing about the death of a man

Detection of Colchicin

Aqueous colchicin solutions, especially in presence of dilute mineral acids, have a yellow color Consequently if a few drops of

¹ A Windaus Investigations upon Colchicin I and II Sitzungsber. d. Heidelberger Akad d Wiss Jg 1910 and 1911

dilute hydrochloric or sulphuric acid are added to a nearly colorless colchicin solution, it becomes more or less intensely yellow. Unless the ether residue has this characteristic, colchicin is absent.

Colchicin and colchicein give the same reactions

1. **Tannic Acid Test.**—This reagent will precipitate colchicin from aqueous solution, if not too dilute, as white flocks. This test, however, is not characteristic of colchicin.

2. **Nitric Acid Test.**—Nitric acid (sp. gr. 1.4 = 66 per cent) dissolves colchicin with a dirty violet color that soon changes when stirred to brownish red and finally to yellow. Addition of dilute sodium or potassium hydroxide solution, until the reaction is alkaline, produces an orange-yellow or orange-red color.

3. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves colchicin with an intense yellow color. A drop of nitric acid, or a particle of potassium nitrate, added to such a solution produces a green, blue, violet and finally a pale yellow tone. Excess of potassium hydroxide solution will now develop an orange-red color. Erdmann's and Froehde's reagents dissolve colchicin with an evanescent blue to violet color soon passing into yellow.

4. **Hydrochloric Acid Test.**—Concentrated hydrochloric acid dissolves colchicin with an intense yellow color. Add 2–6 drops of ferric chloride solution and heat the mixture 2–3 minutes in a test-tube until half the liquid has evaporated. A deep olive-green color appears, becoming darker on cooling and rendered permanent by dilution with water. Finally shake the solution with a few drops of chloroform. The latter becomes yellowish brown, or garnet-red, the aqueous solution retaining its green color. This test given by Zeisel is due to saponification of methoxyl-groups, formation of phenols, and reaction of the latter with ferric chloride.

Fühner's¹ Procedure for Zeisel's Test

Fühner recommends a combination of chemical and biological tests for the forensic detection of colchicin. In Zeisel's test, it is best to bring about preliminary cleavage of colchicin for the color reaction by warming with about 5 drops of 20 per cent. hydrochloric acid, because if hydrolytic cleavage of colchicin is carried beyond the colchicein stage, ferric chloride does not produce a red product.

¹H. Fühner. *Toxicological Detection of Colchicin*. Arch. f. exper. Path. u. Pharm. 63 (1910), 357.

soluble in chloroform. According to Fuhner, white mice may be used in the biological detection of minute quantities of colchicin. After 24 hours they die with severe diarrhoea from 0.1 mg. of colchicin-Boehringer. Immunity of frogs to colchicin, as to tetanus toxin, may be destroyed by warmth. At 30-32° a frog will die in 2-4 days from 0.1 mg. of colchicin-Boehringer. Toxicity of colchicin for frogs at this temperature, as compared with room temperature, is 500 times greater, so that frogs also may be used in the biological detection of colchicin. Colchicin administered subcutaneously is partly eliminated by frogs in an active form in urine. In isolating colchicin from the organism according to Fühner, the gastro-intestinal contents together with the faeces of the poisoned animal are extracted with alcohol. The residue from the extract in aqueous solution is extracted with petroleum ether to remove fat and then with chloroform. The residue from this chloroform solution is tested biologically.

Material for Examination.—The best material to use for the detection of colchicin in suspected poisoning is first vomitus, gastric and intestinal contents, and faeces; and then blood, kidneys and urine. Colchicin resists putrefaction for 3-6 months, since it is said to act as a preservative.

Baumert (see Ptomaines, page 457) isolated a cadaveric colchicin from a 22 months old cadaver by extracting the aqueous tartaric acid solution with ether. Like colchicin it dissolved very readily in chloroform. The yellow, amorphous residue had a distinctly bitter as well as sharp taste. Concentrated sulphuric, dilute hydrochloric and nitric acids, dissolved it with yellow color. Toward concentrated nitric acid its behavior was like that of colchicin. Unlike colchicin, however, this ptomaine did not give Zeisel's test.

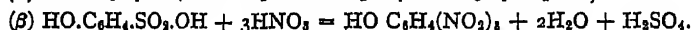
Purification of Colchicin Residue from Ether or Chloroform Extract

To isolate colchicin as pure as possible from the yellow residue, extract with warm water. Filter the solution and extract when cold first with petroleum ether. This will remove fatty, resinous and colored impurities but not colchicin. Then extract with chloroform. Or precipitate colchicin from not too dilute aqueous solution, with tannic acid. Collect this precipitate upon a filter and wash with cold water. Mix the moist precipitate with freshly precipitated, washed lead hydroxide. Dry the mixture, grind to a powder and extract with chloroform. Evaporation of the solvent will leave nearly pure colchicin as a yellow, varnish-like mass that will give the colchicin reactions, especially Zeisel's test, very well.

PICRIC ACID

Picric acid is 2,4,6-trinitro-phenol, $C_6H_2(NO_2)_3(OH)$ and is formed from many benzene derivatives by nitric acid, for example, from aniline, salicylic acid, salicine, indigo, phloridzine, also from such materials as silk, wool and many resins, particularly aloes and gum benzoin.

Preparation.—Phenol and concentrated sulphuric acid are heated until a test portion gives a clear solution in a little water. The phenol-sulphonic acid formed (α) is dissolved in water and, while the solution is stirred, nitric acid (sp. gr. 1.4 = 61 per cent) is gradually introduced (β). Vigorous nitration begins with copious evolution of red fumes of nitrogen peroxide and is finally brought to completion by warming the mixture upon the water-bath. The mass which becomes crystalline upon cooling is freed from admixed nitric-sulphuric acid in a press or centrifuge and washed with cold water. It is then recrystallized from hot water. Picric acid crystals should not be dried at a temperature above 40° , otherwise decomposition may take place with explosive violence.



Properties.—Picric acid crystallizes from water in light yellow, shining leaflets and from ether in lemon-yellow prisms. It dissolves in 86 parts of water at 15° , forming an acid solution having a strong yellow color and intensely bitter taste. It is soluble in 9 parts of alcohol and in 44 parts of ether. The ether solution is only slightly colored and the chloroform and petroleum ether solutions scarcely at all. This behavior is attributed to the fact that picric acid is scarcely ionized in the two last-mentioned solvents. It melts at 122° and may be sublimed when carefully heated. Picric acid can be titrated with n-potassium hydroxide solution with phenol-phthalein as indicator. In titration it behaves like a strong monobasic acid. Solutions of picric acid act as a direct, permanent yellow dye for such animal materials as wool, silk and skin, but not for materials of vegetable origin, such as cotton and paper. Salts of picric acid, called picrates, usually crystallize well. With salts of many alkaloids, picric acid forms yellow, crystalline precipitates of alkaloidal picrates.

Toxic Action and Elimination.—Picric acid is quite a vigorous poison. Taken internally it produces a striking yellow pigmentation first of the conjunctiva and then of the entire skin, usually designated as "picric acid icterus." Picric acid and its salts like most nitro-compounds decompose red blood-corpuscles forming methaemoglobin. Consequently it is a blood-poison. It is further a convulsive poison, for it irritates the central nervous system causing convulsions. Finally it exercises its power of precipitating proteins in acid solution. This manifests itself by necrotic tissue changes especially in those organs of the body that have an acid reaction, such as the stomach and kidneys. The organism reduces picric to picramic acid, $C_6H_3(NO_2)_2(NH_2)(OH)$, which does not so readily precipitate protein. By thus changing picric acid the organism rids

itself of the poison. In picric acid poisoning the urine has a marked red color owing to formation of picramic acid. Some picric acid passes into the urine unchanged. Elimination is slow. In one case (see R. Kobert "Intoxikationen"), after administration of a single dose of 1 gram of picric acid, its presence in the urine could be recognized for 6 days. The urine was ruby-red, clear, acid, and free from albumin and bile-constituents. Picric acid was also easily detected in the faeces.

Detection of Picric Acid

Material containing picric acid has a more or less yellow or yellowish green color. Aqueous, alcoholic and ethereal solutions have the same color. Finely divided animal material should be extracted several hours under a reflux with alcohol containing hydrochloric acid to decompose compounds of picric acid with albumins and thus bring the acid into solution. Filter and evaporate such an alcoholic extract upon the water-bath. Treat the yellow, yellowish green, or frequently yellowish red or reddish brown residue with warm water and filter the extract. Test the filtrate itself direct for picric acid, or first extract as usual with considerable ether. Tests may then be applied to the residue left by evaporating the ether extract. Picric acid is easily extracted by ether or chloroform from a solution strongly acid with mineral acid but not from one faintly acid. This behavior is in full accord with its slight ionization in ether and chloroform. Consequently only the un-ionized portions, not the ions, of picric acid are susceptible of extraction by these solvents. Addition of hydrochloric or sulphuric acid diminishes, that is, strongly represses ionization of picric acid, thus increasing the ease with which it can be extracted.

Reactions of Picric Acid

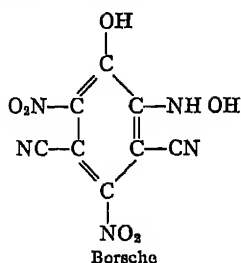
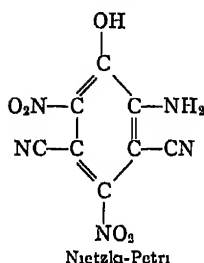
1. **Isopurpuric Acid Test.**—Gently heat (50–60°) an aqueous solution of picric acid with a few drops of saturated aqueous potassium cyanide solution (1:2). The solution will become deep red owing to formation of potassium isopurpurate. Even one mg. of picric acid dissolved in 5 cc. of water (1:5000) gives a deep red color.

Isopurpuric acid does not exist in the free state but is present in this test as the potassium salt. Nietzki and Petri¹ regard isopurpuric acid as a dicyanopicramic acid = 5-oxy-6-amino-2,4-dinitro-isophthalic nitrile, whereas Borsche² considers it a dicyano-dinitro-oxy- β -phenyl-hydroxylamine.

¹ R. Nietzki and W. Petri. Constitution of Isopurpuric Acid. *Ber. d. Deutsch. chem. Ges.* 33 (1900), 1788.

² W. Borsche. Constitution of Meta-purpuric Acid. *Ber. d. Deutsch. Chem. Ges.* 33 (1900), 2995.

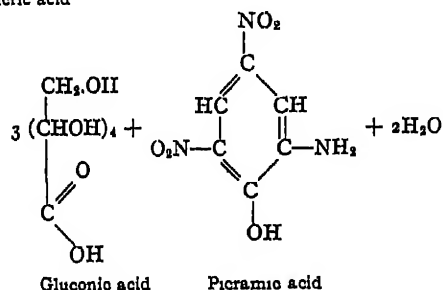
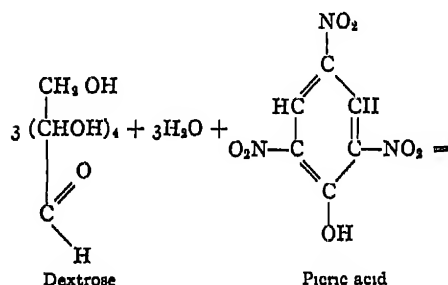
Structural Formulæ of Isopurpuric Acid



2. **Picramic Acid Test.** (a) **With Dextrose.**—Heat an aqueous picric acid solution with 2–3 drops each of aqueous sodium hydroxide and dextrose solution. The solution becomes dark red. Avoid excess of sodium hydroxide solution, otherwise a red color due solely to the action of the alkali upon dextrose will appear.

(b) **With Ammonium Sulphide.**—The same red color appears when a picric acid solution is warmed with a few drops of sodium hydroxide and ammonium sulphide solution.

In both cases picric acid is reduced to picramic acid, 2-amino-4,6-dinitro-phenol. The following equation explains formation of the first oxidation product, gluconic acid, in the test with dextrose:



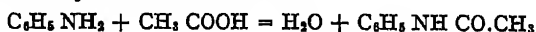
3. **Ammoniacal Copper Test.**—Add a few drops of ammoniacal copper sulphate solution to an aqueous picric acid solution. A yellowish green precipitate, consisting of hexagonal needles having a polarizing action upon light, will appear. A solution of even 1 mg of picric acid in 8 cc of water gives such a precipitate.

4. **Dyeing Test.**—Dissolve the substance containing picric acid in hot water and put threads of wool, silk and cotton in the solution. In a few hours (12–24) remove the threads and thoroughly rinse in pure water. If picric acid is present, the wool and silk will be dyed yellow but not the cotton. In other words, picric acid is not fast upon vegetable fibers like cotton. Picric acid diluted 1:100,000 will still produce a yellow color upon wool.

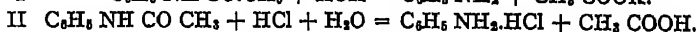
ACETANILIDE

Acetanilide, or antifebrine, is acetylated aniline having the composition $C_6H_5.NH.CO.CH_3$.

Preparation.—Boil aniline and glacial acetic acid together for several hours under a reflux, until a test portion hardens when cooled and stirred. Acetanilide thus formed is recrystallized from hot water.



Acetanilide crystallizes from water in colorless shining leaflets melting at $113-114^\circ$ and having a faint burning taste. It is soluble in 230 parts of cold water, in about 22 parts of boiling water, and in 3.5 parts of alcohol. It is freely soluble in ether and still more so in chloroform. All acetanilide solutions are neutral. Heated to boiling with potassium hydroxide solution (I) and also with fuming hydrochloric acid (II), acetanilide undergoes hydrolytic cleavage into its constituents.



Ether or chloroform will completely extract acetanilide from an aqueous acid solution.

Toxic Action.—As a derivative of aniline, acetanilide possesses the poisonous properties of that amine, though in less degree by reason of the presence of the acetyl-group. Several cases are on record where severe symptoms of intoxication appeared from taking rather large quantities of acetanilide. As yet, however, poisonings with fatal termination have not occurred. If acetanilide were more soluble in water and in the body-fluids, amounts of 28 grams which have been taken at one time would probably have proved fatal. R. Kobert has reported a case where a student took a teaspoonful of the drug. There was stupor, uneasiness, marked cyanosis, and lowering of pulse. A purgative and restorative (stimulant) were used but there was considerable exhaustion for several days. A man who took 2 grams of antifebrine daily for 2 days in succession gave nearly the same picture. In the case of a man 24 years old, who

had taken 12 grams of antifebrine with suicidal intent, there appeared after half an hour dizziness, swimming of objects before the eyes, nausea, increase in acidity, burning in the stomach, cyanosis and syncope. Cyanosis persisted 2 days later, in spite of lavage of the stomach and injection of camphor. Neither albumin nor methaemoglobin was eliminated in the urine. This is the more remarkable, since aniline itself is a typical methaemoglobin-former. In isolated cases a red skin eruption was observed.

Detection of Acetanilide

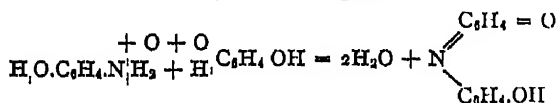
1. **Indophenol Test.**—This test is due to presence of a free, primary, aromatic amino-group (NH_2). Therefore acetanilide must first be hydrolyzed.

Procedure.—Boil acetanilide in a small test-tube with about 4 cc. of fuming hydrochloric acid and evaporate the solution to about 10 drops. Cool and add 2–4 cc. of saturated, aqueous carbolic acid solution. Freshly prepared calcium hypochlorite solution, added drop by drop with good shaking, will produce a more or less intense dirty red-violet color. The color will become deeper, if the mixture is well-shaken for a few minutes. Then carefully add ammonium hydroxide solution as an upper layer. The latter will become a beautiful indigo-blue, whereas the under layer will retain a red color. This blue color is only characteristic of acetanilide and other aniline derivatives when preceded by the dirty red-violet color, since a mixture of an aqueous phenol solution and hypochlorite solution gives a blue color with ammonia. Phenacetine also gives the indophenol test.

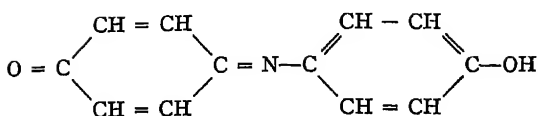
Explanation.—Aniline (see above) produced from acetanilide by hydrolytic cleavage is oxidized by calcium hypochlorite to p-amino-phenol



p-Amino-phenol then reacts with phenol, also under the influence of oxidation, forming with loss of 4 atoms of hydrogen indophenol:



Therefore, in order that an indophenol dyestuff may be formed, only those primary, aromatic amines are available in which there is either an open para-position to the amino-group, or groups in that position that can easily be removed under the influence of oxidation. Such is the case with phenacetine, whereas anaesthesine, $\text{H}_2\text{N.C}_6\text{H}_4.\text{COOC}_2\text{H}_5(1,4)$, does not give the indophenol test. Indophenol is soluble in alcohol with a red color and in sodium hydroxide solution, or ammonia, with a blue color. Its structural formula is as follows



2. **Phenyl-isocyanide Test.**—Boil acetanilide for a few minutes with about 5 cc of alcoholic or aqueous potassium hydroxide solution. Cool, add 2 drops of chloroform and heat again. The offensive odor of phenyl-isocyanide will be developed. Potassium hydroxide hydrolyzes acetanilide, forming potassium acetate and aniline (see above), and the latter with chloroform gives phenyl-isocyanide (see Chloroform, page 60). Phenacetine and other derivatives of p-phenetidine do not give the phenyl-isocyanide test.

3. **Calcium Hypochlorite Test.**—Boil acetanilide for a few minutes with alcoholic potassium hydroxide solution. Dilute with water and extract aniline with ether. Remove the ether extract, evaporate upon a watch-glass, and test the residue for aniline by means of calcium hypochlorite solution or by the phenyl-isocyanide test.

4. **Azo-dyestuff Test.**—The reactions involved in this test require the presence of a free, primary amino-group. Acyl-derivatives of primary aromatic bases, such as acetanilide or phenacetine, must first be hydrolyzed by boiling with concentrated hydrochloric acid (see above).

Procedure.—Boil acetanilide, as described in the indophenol test, for a few minutes with fuming hydrochloric acid. Dilute with 3-5 cc of water, cool well (best in ice), add a few drops of potassium nitrite solution (1:10), render alkaline while cooling with sodium hydroxide solution, and add carefully as an upper layer an alkaline solution of β -naphthol. A red zone will appear at the contact-surface of the two liquids. Different shades of red are given by the different primary aromatic bases.

Explanation.—Nitrous acid converts the hydrochloride of the primary base into a diazonium chloride which sodium hydroxide solution changes to the corresponding diazonium hydroxide. In alkaline solution the latter will couple with a phenol, such as β -naphthol, forming an oxyazo-dyestuff.

(α) $\text{C}_6\text{H}_5 \text{NH}_2 \text{HCl} + \text{HO.NO} = 2\text{H}_2\text{O} + \text{C}_6\text{H}_5.\text{N}.\text{Cl}$ Diazo-benzene chloride.

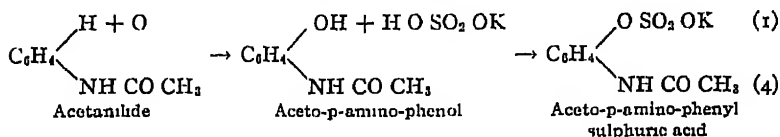
(β) $\text{C}_6\text{H}_5.\text{N}.\text{Cl} + \text{NaOH} = \text{NaCl} + \text{C}_6\text{H}_5.\text{N} = \text{N}.\text{OH}$ Diazo-benzene hydroxide.

(γ) $\text{C}_6\text{H}_5.\text{N} = \text{N}.\text{OH} + \text{H}|\text{C}_{10}\text{H}_7.\text{OH} = \text{H}_2\text{O} + \text{C}_6\text{H}_5.\text{N} = \text{N}.\text{C}_{10}\text{H}_7.\text{OH}$ β -Naphthol-oxyazobenzene.

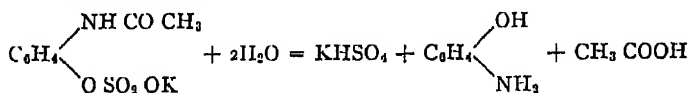
5. Detection of Acetyl Group.—When the amount of material is not too small, the test for acetic ether may be made. Heat for a few minutes in a boiling water-bath, mixed in a small test-tube with concentrated sulphuric acid and some alcohol. If acetanilide is present, the characteristic odor of ethyl acetate will appear upon careful addition of a little water.

Examination of Acetanilide Urine¹

Human urine contains acetanilide as such only after rather large doses have been taken. Most of the acetanilide is oxidized in the human organism to aceto-p-amino-phenol, which like nearly all phenols undergoes conjugation with acid potassium sulphate forming an ethereal sulphuric acid, aceto-p-amino-phenyl-sulphuric acid which is eliminated in the urine.



Part of the aceto-p-amino-phenol undergoes conjugation in the human body with glycuronic acid. These conjugated acids, heated with concentrated hydrochloric acid, are hydrolyzed. One component of the cleavage, p-amino-phenol, gives the indophenol test.



An "acetanilide urine," boiled for a few minutes with concentrated hydrochloric acid, usually gives the indophenol test. But the test is more certain, if p-amino-phenol is first isolated. Boil a considerable quantity of urine (300–500 cc.) for a few minutes with about 10 cc. of concentrated hydrochloric acid under a reflux. Then add excess of sodium carbonate and repeatedly extract the cold urine with large quantities of ether. Distil or evaporate the ether. The residue usually contains p-amino-phenol as a reddish or brownish oil. An aqueous solution of this substance will give the indophenol test.

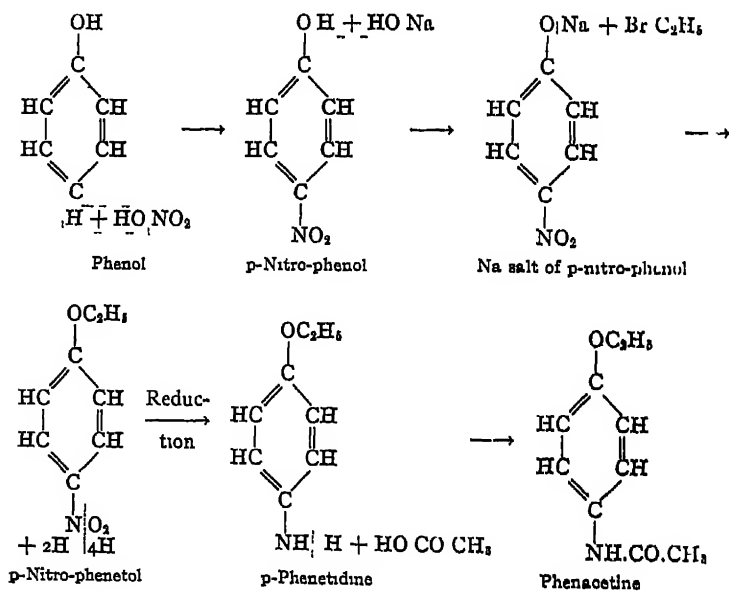
PHENACETINE

Phenacetine, p-aceto-phenetidine, $\text{C}_2\text{H}_5\text{O} \cdot \text{C}_6\text{H}_4 \cdot \text{NH CO CH}_3$ (1, 4), crystallizes in shining leaflets without color, odor or taste, and melts at 134–135°. It is soluble in 1400 parts of cold water, in about 70

¹ To study the behavior of acetanilide in the human organism, take at night twice in the course of 3 hours 0.3 gram of this compound at a dose and examine in the manner described above the urine passed in the next 12 hours.

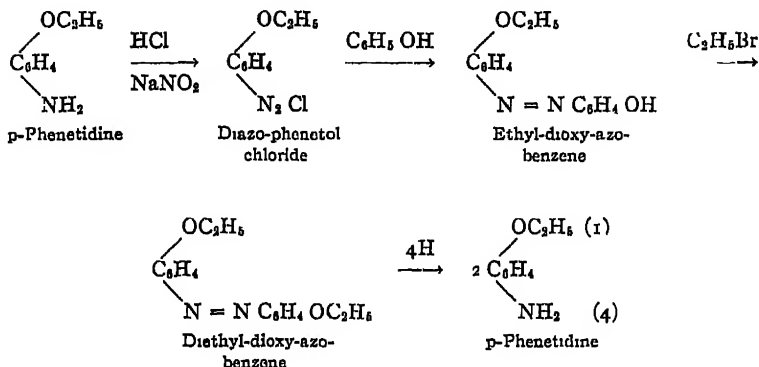
parts of boiling water, 16 parts of alcohol, and freely soluble in ether and chloroform. Its solutions are neutral. Concentrated sulphuric acid dissolves it without color. Phenacetine can be easily and completely extracted by ether or chloroform from an acid, aqueous solution.

Preparation.—Gradual addition of crystallized phenol to cold dilute nitric acid (sp gr 1.11 = 17.5 per cent) results in the formation of a mixture of o- and p-nitro-phenol. Since the o-compound is volatile with steam, complete separation of the two products is possible by steam distillation. Residual p-nitro-phenol is converted into its sodium salt by sodium hydroxide solution and then into p-nitro-phenetol by ethyl bromide. This product is reduced by nascent hydrogen from tin and hydrochloric acid to p-amino-phenetol = p-phenetidine, which is finally boiled with glacial acetic acid and converted into aceto-p-phenetidine = phenacetine.



According to Riedel, p-phenetidine is diazotized in hydrochloric acid solution by sodium nitrite and converted into diazo-phenetol chloride. The latter coupled in alkaline solution with phenol gives ethyl-dioxy-azo-benzene which upon ethylation by ethyl bromide and sodium hydroxide solution yields diethyl-dioxy-azo-benzene. This compound reduced by nascent hydrogen from tin and hydrochloric acid gives two molecules of p-phenetidine. Half of this final product is boiled for several hours with glacial acetic acid and converted into

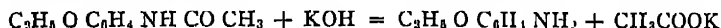
phenacetine, whereas the other half is passed again through the reactions just described.



Physiological Action.—In man phenacetine does not bring about decomposition of blood with formation of methaemoglobin, when the dose required as a febrifuge or antineuralgic is not exceeded. In one instance Krönig observed occurrence of death accompanied by very severe decomposition of blood in the case of a 16 year old compositor who had taken 1 gram of phenacetine. According to Lewin, phenacetine may give rise to cyanosis with and without collapse. A woman had taken 2 grams of phenacetine in 3 hours. Although the symptoms that appeared after the first gram were mainly nervous, such as dizziness and trembling, after the second gram taken 3 hours later there was a feeling of intense cold, severe cyanosis of the fingers, hands, lips and cheeks, deep blue coloration of the skin with cold sweat, feeling of anxiety and dyspnoea. This coloration still persisted four hours later. Even the palate and mucous surfaces were cyanotic. Not until 12 hours had elapsed did this condition begin to abate. In this as in every case where blood decomposition occurs, notwithstanding the blue coloration due to methaemoglobin, icterus could be detected locally. As often happens in methaemoglobinaemia, the blood in phenacetine intoxication gives evidence of changes in red blood-corpuscles from which the pigment has either wholly disappeared or has agglomerated in one place. The secondary effects upon the skin also deserve mention. Exanthema of the skin may occur, chiefly in the extremities, in persons with and without fever. Red spots that coalesce and give rise to burning may appear and fade out on the following day. Swellings of the skin may also occur. After a dose of 2 grams of phenacetine, taken at one time in the evening, there was observed in the case of a man suffering from ischias an oedematous swelling of both the lower eyelids. After further administration of 3 grams in two doses, the same kind of swelling appeared on the forehead, the bridge of the nose, and the upper part of the cheeks. Moreover all the fingers were swollen to such an extent that they could not be bent at the joints (Lewin).

Detection of Phenacetine

As a substituted acid-amide, phenacetine undergoes hydrolytic cleavage into p-phenetidine and acetic acid, when heated with potassium hydroxide solution or concentrated hydrochloric acid:



1. **Oxidation Test.**—Boil phenacetine for several minutes with about 3 cc. of concentrated hydrochloric acid. Dilute with water to about 10 cc and filter when cold. Addition to the filtrate of a few drops of 3 per cent chromic acid solution will produce a ruby-red color. In this test acetanilide gives a yellow color gradually turning green. Strong chlorine water may be substituted for chromic acid.

2. **Indophenol Test.**—Treated in the manner described for acetanilide (see page 123), phenacetine gives a very good indophenol test.

3. **Azo-dyestuff Test.**—After preliminary boiling with concentrated hydrochloric acid, phenacetine gives a distinct test due to formation of an azo-dyestuff, as described under acetanilide (see page 124)

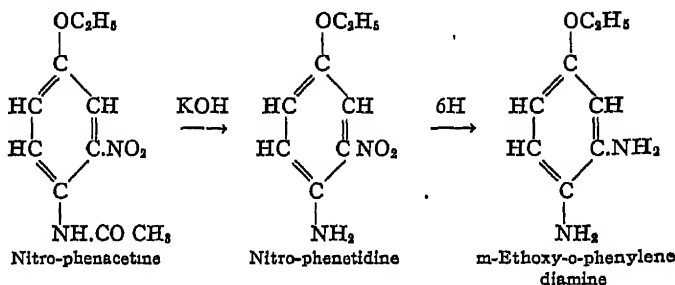
4. **Autenrieth-Hinsberg Test.**¹ (a) **With Dilute Nitric Acid.**—Heat phenacetine to boiling with dilute nitric acid (10–12 per cent). It dissolves with a yellow to orange-red color. If the solution is saturated, it will deposit upon cooling long, yellow needles of mono-nitro-phenacetine, $\text{C}_6\text{H}_3(\text{NO}_2)(\text{OC}_2\text{H}_5)(\text{NH CO.CH}_3)$, melting at 103° . This test is delicate and characteristic of phenacetine, when nitro-phenacetine can be obtained in crystals and sufficiently pure for a determination of melting-point. Acetanilide and antipyrine give colorless solutions when warmed with dilute nitric acid.

(b) **With Concentrated Nitric Acid.**—A few drops of concentrated nitric acid added to phenacetine produce a yellow to orange color. Part of the phenacetine is dissolved with the same color. Warming, especially upon addition of water, dissolves it completely and nitro-phenacetine crystallizes in yellow needles as the solution cools.

When phenacetine is nitrated, the nitro-group takes the position ortho to the acetamino-group. This is shown by the fact that nitro-phenacetine, when heated with alcoholic potassium hydroxide solution, gives nitro-phenetidine crystallizing in ruby-red needles. The latter upon reduction with nascent

¹ W. Autenrieth and O. Hinsberg. Contribution to the Knowledge of Phenacetine and m-Ethoxy-o-Phenylene-Diamine. Arch. d. Pharm. 229 (1891), 456

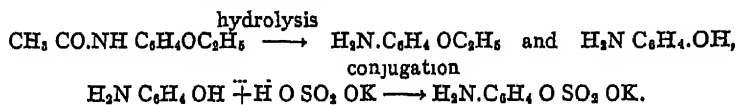
hydrogen from zinc dust and potassium hydroxide solution is converted into an ortho-diamine



5. **Differentiation Test.**—Phenacetine differs from acetanilide in not giving the phenylisocyanide test, when warmed with chloroform in presence of potassium hydroxide solution.

Phenacetine Urine

After rather large doses, phenacetine sometimes appears in the urine unchanged. Used in moderate doses, phenacetine undergoes hydrolytic cleavage in the human body with formation of p-phenetidine and p-amino-phenol. These compounds are eliminated in the urine either by themselves or as conjugates of sulphuric and glycuronic acid.



Therefore phenacetine-urine usually gives the indophenol and azo-dye test. These tests are always given, if the urine has previously been boiled with hydrochloric acid.

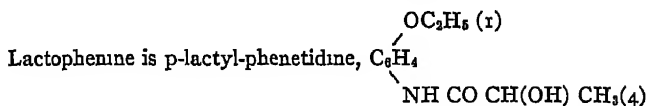
Procedure.—Heat 10–20 cc of urine to boiling with a few drops of concentrated hydrochloric acid. Cool (best with ice), add 2–3 drops of 10 per cent. sodium nitrite solution, shake, then add 2 drops of 5 per cent. alcoholic α -naphthol solution, and finally render alkaline with sodium hydroxide solution. If p-phenetidine or p-amino-phenol is present, a red color will appear and change to a beautiful dark cherry-red upon addition of hydrochloric acid. Edlfsen¹ prefers the alcoholic to the alkaline solution of α -naphthol. The burgundy-red to brown-red color, sometimes appearing when ferric chloride, calcium hypochlorite, or chromic acid (3 per cent.) is added to phenacetine-urine, may be due to presence of p-phenetidine.

Phenacetine-urine is frequently intensely yellow and exhibits in consequence of presence of conjugated glycuronic acid laevo-rotation and reduction of Fehling's solution. It differs from urine containing sugar in not fermenting. Detection of p-amino-phenol is more certain, if it is first isolated from urine in the manner

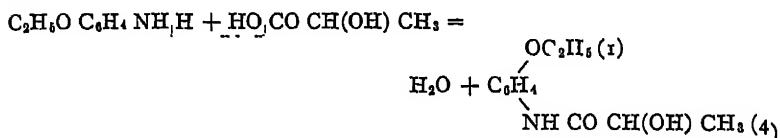
¹ G. Edlfsen: Detection of Phenetidine in Urine. Zentralblatt f. inn. Med. 21 (1900), 2.

described for acetanilide-urine (see page 125) and then examined by the indophenol and azo-dye test.

LACTOPHENINE



Preparation—Heat p-phenetidine lactate, that is, lactic acid and phenetidine, or p-phenetidine with lactic anhydride or the ethyl ester of lactic acid at 150–180°



Lactophenine forms small, colorless, odorless crystals having a bitter taste and melting at 117–118°. It is soluble in about 330 parts of cold and in 55 parts of boiling water, and in 85 parts of alcohol. These solutions have a neutral reaction.

Toxic Action.—Following use of lactophenine, icterus has been observed with relative frequency. In 9 out of 20 cases icterus appeared after use upon the average of 27 grams distributed over 9 days (S. Laache). The patients were adults, vigorous and usually free from fever. In 77 per cent of the cases, illness was accompanied by an acute, transient fever. The urine usually had a deep brown color, and in nearly all positive cases gave Gmelin's test for bile-pigment. This test is made by putting in a test-tube 5 cc of concentrated nitric acid, containing 2 drops of fuming nitric acid in 100 cc, and adding the same quantity of urine, best from a pipette, as an upper layer. If bile-pigment is present, an emerald-green ring appears at the contact-surface of the two liquids. Gradually this ring extends upward and by degrees at the lower part becomes blue, violet-red and yellow. Only the green ring is characteristic of bile-pigment.

According to Rosenbach, pass rather a large quantity of the given urine through a small filter. Allow it to drain well, then open the filter on a glass plate, and spot on the inner side with nitric acid containing some fuming acid. In this manner concentric rings of yellow-red, red, violet, blue and green colors extending outward are obtained.

Skin eruptions may appear, attended with discomfort, temperature in the head, ague, fever, increased pulse and headache. Occasionally blue coloration of the skin probably because of methaemoglobinaemia, with or without collapse, has been observed. The pulse may be quickened and intermittent. Dizziness with disturbances of sight, cloudiness of vision and slight loss of consciousness may produce partial collapse (Lewin).

Detection of Lactophenine

Boiled with hydrochloric acid, lactophenine undergoes hydrolytic cleavage with formation of p-phenetidine which gives the indophenol and azo-dye

test To differentiate it from phenacetine, the following tests given only by lactophenine may be used

1 Ferric chloride imparts a reddish brown color to an alcoholic solution of lactophenine.

2 Solution of lactophenine in dilute sulphuric acid even in the cold quickly discharges the color of dilute potassium permanganate solution If this mixture is gently warmed, odor of acetaldehyde can be detected This is due to oxidation of lactic acid formed by hydrolytic cleavage.



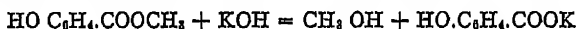
3 Bromine water added to an aqueous solution of lactophenine produces a white precipitate

Urine.—To detect p-amino-phenol with certainty, this compound should be isolated from lactophenine-urine in the manner described for acetanilide-urine (see page 125) and then detected by the indophenol and azo-dye test

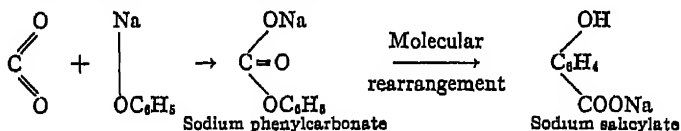
SALICYLIC ACID

Salicylic acid, or o-oxy-benzoic acid, $\text{HO.C}_6\text{H}_4.\text{COOH}$ (1, 2), is fairly wide-spread in the plant kingdom in the free state, also as salicylic aldehyde and methyl ester, and finally as the glucoside salicine This acid was first isolated from the flowers of *Spiraea Ulmaria*. As the methyl ester, $\text{HO.C}_6\text{H}_4.\text{COOCH}_3$, salicylic acid occurs in various ethereal oils and as the chief constituent of oil of wintergreen (*Gaultheria procumbens*).

Preparation.—1 Formerly oil of wintergreen was the source of this acid When heated with potassium hydroxide solution, it was decomposed into methyl alcohol and potassium salicylate. Acidification of the latter with hydrochloric acid precipitated salicylic acid which finally was crystallized from hot water:



2. At the present time salicylic acid is prepared from carbolic acid by the method of R. Schmitt Phenol is dissolved in strong sodium hydroxide solution. Perfectly dry sodium phenolate is obtained by evaporating this solution This compound is placed in an autoclave, cooled, and saturated under pressure with carbon dioxide The first product of this reaction is sodium phenyl-carbonate. When this is heated at $120-130^\circ$, it undergoes molecular rearrangement and is converted into sodium salicylate frequently used in medicine



Properties.—Salicylic acid crystallizes from water in long, white needles having a sweetish, acidulous and rather harsh taste. It is soluble in about 500 parts of cold and in 13 parts of boiling water, and freely soluble in alcohol (1:2), ether

(1:2), chloroform, acetone and carbon disulphide. It melts at 157° . When heated carefully and slowly in the water-bath, salicylic acid sublimes in fine needles. On the other hand, quick heating decomposes it in large part into phenol and carbon dioxide.



Salicylic acid may also be easily volatilized with steam. Concentrated sulphuric acid dissolves pure salicylic acid in the cold without color and without decomposition. The lead and silver salts of this acid are soluble in water with difficulty. Consequently lead acetate will precipitate lead salicylate, $\text{Pb}(\text{OOC.C}_6\text{H}_4.\text{OH})_2$, from neutral solutions. This salt is white, crystalline and soluble in hot water. It crystallizes unchanged as the hot solution cools. Silver nitrate precipitates white silver salicylate, $\text{HO.C}_6\text{H}_4.\text{COOAg}$.

Physiological Action.—Salicylic acid produces local effects and those resulting from absorption. Locally it acts like a phenol but considerably more feebly than carbolic acid, coagulating albumin and so killing living protoplasm when in very intimate contact. If salicylic acid in pulverulent form is applied to mucous surfaces, it produces white eschars as well as inflammation and necrotic sloughing as a result of this action. Absorption of salicylic acid takes place from all mucous surfaces with such rapidity that it can be detected in the urine even after 15 minutes. The effects of absorption appear in the blood-vessels, then in the central nervous system and kidneys. They arise from injury to protoplasm as in local action. In the kidneys they result from damage to the secreting epithelium (Kobert). Elimination of salicylic acid in the urine begins in the first hour and ends as a rule within 3 days. In addition to elimination in the urine, this acid also leaves the body in perspiration, bile and milk. According to Filippi (*La clinica moderna* 1900), part of the salicylic acid is said to be eliminated in the synovial fluid of the joints. The brain and spinal cord also withdraw salicylic acid from the blood. Several cases have been described in the literature where severe symptoms of poisoning appeared, following doses of 15, 22 and 50 grams of sodium salicylate. They gave rise to emesis, unconsciousness, delirium, difficulty in hearing, lowering of visual power (mydriasis), albuminuria, fall of temperature, weakness and irregularity of pulse. Recovery, however, took place after a few days. In treatment of articular rheumatism with salicylic acid, the following effects have been repeatedly observed: nausea, emesis, ringing in the ears, faintness of vision, skin eruptions, haemoglobinuria, haematuria. In urinary sediments have been found cylinders, epithelial cells of the urinary tubules, and white blood-corpuscles. Addition of salicylic acid as a preservative to foods and condiments is prohibited.

Detection of Salicylic Acid

1. **Ferric Chloride Test.**—Addition of a few drops of ferric chloride solution to an aqueous or alcoholic solution of salicylic acid produces a blue-violet color. If the solution is very dilute, the color is more of a red-violet. By this test salicylic acid is shown to be a derivative of phenol. Excess of the reagent affects the delicacy of the test.

Addition of hydrochloric acid changes the violet color to yellow. Presence of organic acids, such as citric, lactic and tartaric acid, also interferes more or less with this test. This test is not given, if caustic alkalis or alkaline carbonates are present.

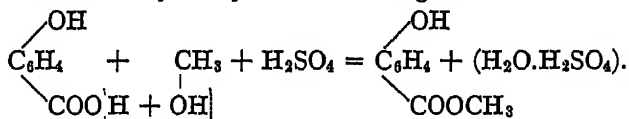
2. **Millon's Test.**—If an aqueous salicylic acid solution is warmed with Millon's reagent, a deep red color will appear.

3. **Bromine Water Test.**—In strong excess this reagent produces a yellowish white, crystalline precipitate of tribromo-phenyl hypobromite (see page 44) even with very dilute salicylic acid solutions:



This precipitate should be examined microscopically and its melting-point determined, after it has been filtered and dried upon a porous plate or in a vacuum-desiccator. It melts at $131-132^\circ$ with evolution of gas.

4. **Methyl Ester Test.**—If salicylic acid is warmed, best in a water-bath, with methyl alcohol and concentrated sulphuric acid, the characteristic odor of methyl salicylate can be recognized:



5. **Melting-point Test.**—If the quantity of salicylic acid is not too small, dissolve the ether residue in very little boiling water, shake the hot solution with animal charcoal, filter hot, and allow to crystallize. Determine the melting-point of the crystalline needles, when dry, both direct and after mixing with an equal quantity of pure salicylic acid. In case the quantity permits, make the tests for salicylic acid in 1 to 4. An aqueous distillate should be extracted with a little ether, the ether evaporated, and the residue tested for salicylic acid as described.

Detection of Salicylic Acid in Presence of Simple Phenols

Monacid phenols, such as carboic acid and the cresols, also give the tests described above in 1 to 3. Separation of carboxylic acids from phenols may be brought about by a cold solution of sodium carbonate which forms salts with acids but not with phenols. To detect salicylic acid in presence of phenols with greater certainty, acidify the material, if neutral or alkaline, with hydrochloric acid, cool and make alkaline with sodium carbonate solution, and completely extract with ether any phenols that may be present. Render the

aqueous solution, containing salicylic acid as the sodium salt, acid with hydrochloric or sulphuric acid. Extract free salicylic acid with ether and test the ether extract.

Directions are given in Chapter V of this book concerning detection of salicylic acid in beer, wine, milk, fruit-juices, meat, sausage-materials, etc., according to the official methods, and also maltol.

Quantitative Estimation of Salicylic Acid as Tribromo-phenyl Hypobromite

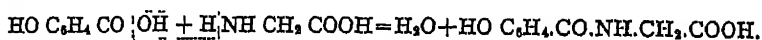
(Autenrieth and Beuttel¹)

Place the aqueous solution of salicylic acid in a glass-stoppered flask, add excess of saturated bromine water and shake. The acid is completely precipitated as tribromo-phenyl hypobromite. The solution in the end should be reddish brown. It should stand 12-24 hours and be shaken frequently. Collect the precipitate of tribromo-phenyl hypobromite in a weighed Gooch crucible and dry to constant weight in a vacuum-desiccator (Fig 13, page 96) over sulphuric acid. The quantity of salicylic acid may be calculated from the weight of precipitate according to the following proportion:

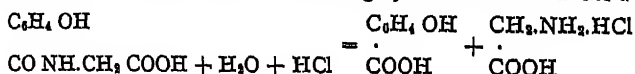
$C_6H_2Br_4O(409.86) : C_7H_5O_3(138.05) = \text{Weight of Precipitate} : x$.
Consequently the weight of the precipitate should be multiplied by 0.337.

Detection of Salicylic Acid in Urine²

Salicylic acid forms a conjugate with glycocholic acid, supplied by the organism, and is changed in the human body, in part at least, into salicyluric acid which is eliminated in the urine together with larger quantities of unchanged salicylic acid.



Such urine gives a violet color with ferric chloride solution. Both salicylic and salicyluric acid give this test. To decompose salicyluric acid into its constituents, heat the acid for half an hour with fuming hydrochloric acid under a reflux:



To isolate unchanged salicylic acid, acidify 500-1000 cc. of urine with dilute sulphuric acid and repeatedly extract with ether. Remove the ether from the

¹ W. Autenrieth and Fr. Beuttel. Estimation of Phenol, Salicylic Alcohol and Salicylic Acid. Arch. d. Pharm. 248 (1910), 112.

² To study the behavior of salicylic acid, take 1.5 grams of sodium salicylate at night in the course of several hours and examine, as described, the urine passed in the next 12-24 hours.

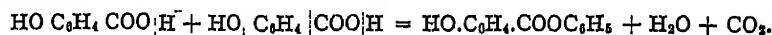
urine in a separating funnel and shake vigorously with excess of sodium carbonate solution. Salicylic acid passes into the aqueous solution, whereas phenols, coloring-matter and other interfering substances remain in the ether. Acidify the sodium carbonate solution with hydrochloric acid and extract salicylic acid with ether. When the ether is distilled or evaporated, it will usually leave this acid in the form of crystals. If necessary, it may be recrystallized from a little boiling water and freed from color by boneblack. A large part of the salicylic acid taken internally can be recovered from human urine. After 3 days elimination is complete.

E. Späth's Procedure.—When a urine gives an uncertain test with ferric chloride, probably very little salicylic acid is present, or substances are causing interference. Evaporate a considerable quantity of the urine upon the water-bath to a small volume, acidify with dilute sulphuric acid, and extract with twice the volume of a mixture of 3 parts of low-boiling petroleum ether and 2 parts of chloroform. According to Späth, this solvent is to be preferred to ether in the examination of foods and condiments, because it contains practically no water and for that reason does not take up substances, such as tannins, that interfere with the ferric chloride test. Pass the solvent through a dry paper, add 1 cc. of water and only a drop of a ferric chloride solution so dilute that the yellow color is just visible, and shake. If salicylic acid is present, the aqueous solution will have a violet color.

SALOL

Of the large number of derivatives of salicylic acid, used medicinally as substitutes for free salicylic acid and its sodium salt, only salol and aspirine have been considered in this book. These two preparations have come into very wide use in medicine. Both possess the valuable therapeutic properties of salicylic acid without its injurious action, that is, without irritating the stomach or exerting other unpleasant secondary effects.

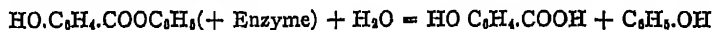
Preparation.—Salol is formed by heating dry salicylic acid for 4 hours in an atmosphere of carbon dioxide at 220–230°.



Consequently salol is the phenyl-ester of salicylic acid.

Salol is a white, crystalline powder having a faint aromatic odor and taste. Its melting-point is approximately 42°. It is almost insoluble in water; soluble in 10 parts of alcohol, 0.3 part of ether, and also in chloroform.

Physiological Properties.—Since salol is an ester, at the temperature of the body, in contact with the secretion of the pancreas, with the living mucous membrane of the intestines and other living tissues, it undergoes decomposition into phenol and salicylic acid.

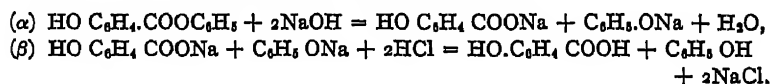


Therefore symptoms of intoxication, due to both these cleavage-products, may appear. Both occur in the urine. For this reason salol-urine possesses the properties of carbolic-urine. It gradually acquires an olive-green color, becoming finally brown and brown-black. A man 40 years old who had taken

22.5 grams of salol in 4 days had a strong albuminuria and severe pain in the region of the kidneys. A girl 22 years old after taking 8 grams of salol became drowsy, fell into a coma, and the perspiration had an aromatic odor. Death ensued on the third day. The autopsy revealed acute and profound degeneration of the epithelium of the kidneys. Where individuals have used salol externally in the form of ointments, typical intoxications, especially diseases of the skin, have appeared. In two instances where powdered salol was blown into the nose, eczema was developed in the nostrils and upon the upper lip. In a third case the eczema extended over a large part of the face. A vaseline ointment containing 10 per cent of salol was responsible for this result.

Detection of Salol

An alcoholic solution of salol, but not an aqueous, filtered extract, takes on a dirty violet color with very dilute ferric chloride solution. If 0.2–0.3 gram of salol is brought into solution by warming with as little sodium hydroxide solution as possible, sodium phenolate and sodium salicylate are formed. Crystals of salicylic acid separate and the odor of phenol can be recognized, when this solution is cooled and acidified with hydrochloric acid.

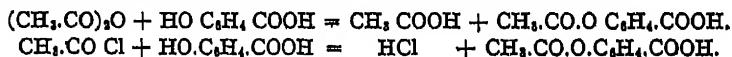


To separate the two products of this reaction, extract first with ether and then shake the ether solution with sodium carbonate solution. Phenol remains dissolved in the ether, whereas salicylic acid goes into the sodium carbonate solution as the sodium salt. If the latter is acidified with hydrochloric acid, ether will now extract only salicylic acid. The residues obtained by distilling or evaporating these two ether solutions may be tested in the manner already described for phenol and salicylic acid.

Similarly salol-urine may be acidified with hydrochloric acid and extracted with ether. The residue from this ether solution can be tested for the two cleavage-products, that is, phenol and salicylic acid, formed in the animal organism, or for salicylic acid resulting from salicylic acid.

ASPIRINE

Aspirine, or aceto-salicylic acid, $\text{CH}_3\text{CO.O C}_6\text{H}_4\text{.COOH}$ (1, 2), is formed by the action of acetic anhydride or acetyl chloride upon salicylic acid.



Aspirine is a white, inodorous, crystalline powder having a sweet, acidulous taste and melting at about 137° . It is soluble in 100 parts of water, 4.5 parts of alcohol, 10 parts of ether, and 26 parts of chloroform. An aqueous aspirine solution has an acid reaction. Ether extracts aspirine from an acid aqueous solution. Heated with potassium or sodium hydroxide solution, or even with water, it is hydrolyzed into salicylic acid and acetic acid:



Physiological Properties.—Aspirine irritates the mucous membrane of the stomach but slightly and like salol reaches the intestines without decomposition. There it is only slowly resolved into its two components. The remainder is decomposed after absorption. Its elimination is slower than that of sodium salicylate. The urine contains salicylic and salicyluric acid. Poisonings from aspirine with fatal outcome have probably not occurred. Repeatedly, mild toxic symptoms have been observed after doses of 0.5–1 gram of aspirine, that is, oedematous swellings, especially of the eyelids, and of the forehead and scalp. But they have usually disappeared quickly. Severer symptoms of poisoning have been observed after the administration of aspirine to children: drowsiness, coma, emesis, acetone in the breath and urine.

Detection of Aspirine

Heat 0.2–0.5 gram of aspirine for a few minutes with a little sodium hydroxide solution, cool, best by setting the test-tube in ice-water, and acidify with dilute sulphuric acid. After some time filter off the salicylic acid that crystallizes out and apply the tests already described for its recognition. The second component of the hydrolysis of aspirine, that is, acetic acid, can usually be recognized by its odor in the filtrate from salicylic acid. To detect it with greater certainty, warm the filtrate with some alcohol and concentrated sulphuric acid. The odor of acetic ether will be developed. To detect salicylic and salicyluric acid in urine, test a portion with ferric chloride. If the result is not convincing, proceed according to the method (see page 134) employed in detecting salicylic acid in urine.

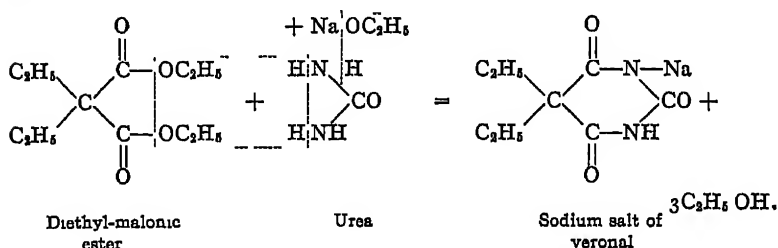
VERONAL

Veronal, or barbital (name adopted by the Federal Trade Commission for the substance formerly sold under the protected name "Veronal"), is C-diethyl-barbituric acid, C-diethyl-malonyl-urea, $C_8H_{12}O_4N_2$. It crystallizes from hot water in large, colorless, spear-shaped crystals melting at 191° (corrected) and is soluble in 146–147 parts of water at 20° and in 15 parts at 100° . Veronal is also freely soluble in hot alcohol and acetone. It dissolves with difficulty in cold ether, benzene and chloroform. Heated above its melting-point, veronal sublimes in long, shining needles almost without decomposition. An aqueous veronal solution has a bitter taste and shows a very faint acid reaction with sensitive blue litmus paper. Veronal dissolves easily in caustic alkalis, ammonia, and in calcium or barium hydroxide solution, but less easily in cold alkaline carbonate solutions. From such solutions, provided they are not too dilute, acids precipitate veronal unchanged and usually crystalline. Of the veronal salts, the sodium salt, $C_8H_{11}O_4N_2Na$, crystallizes best. It may be prepared by dissolving veronal in the calculated quantity

of caustic soda solution free from carbonate, and then evaporating this solution with exclusion of carbon dioxide, or by adding alcohol until turbidity appears. In both cases the sodium salt of veronal, also called "Medinal," separates as splendid shining crystals.

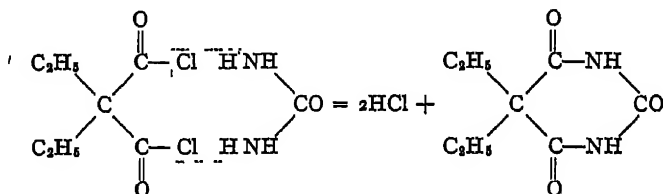
Preparation by Fischer and Diltney¹

(a) From diethyl-malonic ester by condensation with urea in presence of sodium ethylate.



Dissolve metallic sodium (32 parts) in absolute alcohol (600 parts) and when cold add diethyl-malonic ester (100 parts). Dissolve in this mixture with heat finely powdered urea (40 parts). Heat 4-5 hours in an autoclave at 105-108°. The sodium salt of veronal is precipitated even from the hot solution as a colorless, crystalline mass. Cool, filter with suction and wash with alcohol. Dissolve the crystals in water and acidify with concentrated hydrochloric acid. Veronal thus precipitated is pure when recrystallized from water.

(b) From diethyl-malonyl chloride by condensation with urea.



Heat diethyl-malonyl chloride (3 parts) upon the water-bath for 20 hours with finely powdered, dry urea (2 parts). Considerable hydrogen chloride is given off toward the end and a solid mass finally remains that yields pure veronal upon crystallization from hot water. The yield is 70 per cent. of the theoretical amount.

Toxic Action.—Veronal does not cause decomposition of the blood and in the usual medicinal doses (0.5-1 gram) does not appear to act strongly upon the heart. Cumulative action has been noted only in rare instances. In rather large doses, however, veronal may cause serious intoxication with fatal termina-

¹ E. Fischer and A. Diltney. C-Dialkyl-barbituric Acids and the Ureids of Dialkyl-acetic Acids. *Annalen d. Chemie* 335 (1904), 334.

tion The opinion in general appears to be that 10 grams of veronal is a fatal dose for adults The symptoms of acute veronal poisoning resemble those of morphine poisoning abnormally deep sleep, prolonged unconsciousness, cyanotic face, feeble respiration, retching, tetanus-like convulsions, extremities cold and sluggish, contracted and unreactive pupils make up the usual picture of acute veronal poisoning. The following case shows the course of a veronal- (medinal-) poisoning

Autenrieth¹ recovered from the urine of a girl, who had taken 20 tablets each of 5 gram of medinal at a dose, 3 2 grams of nearly pure veronal After 36 hours of sleep, 400 cc of urine were drawn from the bladder by catheter The physician, who had charge of the girl, gave the author the following record of the case:

"Twenty medinal tablets were dissolved in water and taken at a dose About 10 hours later there was deep coma, complete anaesthesia, pupils highly contracted, showing scarcely any reaction, otherwise no reflexes; general paresis; pulse 120, low but regular; respiration 32, regular Later on pulse up to 150, regular, respiration up to 75, very feeble, somewhat irregular (30 to 34 hours after the poisoning) Following injections of camphor, pulse and respiration improved! After the effect of the camphor had worn off, improvement disappeared After 70 hours there was still only a very slight quickening of pulse and respiration After 44 hours there was a very faint corneal reflex. After 54 hours there were slight muscular movements Unconsciousness lasted for 60 hours! After 66 hours consciousness was tolerably clear. Temperature 38.6 to 39.1°, quick drop to 36.8°, following a spontaneous stool after 54 hours Retention of urine during 54 hours Elevated, urticaria-like, red spots appeared upon the inner surface of the legs upon the impressions of the condyles These did not completely disappear for a week Upon return of consciousness after 66 hours pressure in the head, slight stupor which, however, quickly passed away During 4-5 days there was muscular uneasiness and pains in the limbs After 5 days the urine was again free from albumin "

This case shows that veronal is a sure and also tolerably safe hypnotic The 20 medinal tablets, taken in aqueous solution at one dose by this 18 year old girl, corresponded to 8 grams of veronal. On the other hand, 2 cases of suicide from veronal are known. In one case 11 grams and in the other 15 grams of veronal were taken at a dose In the latter case after 15 minutes there was unconsciousness and contraction of the pupils Atropine again dilated the pupils but otherwise was of no avail. Death ensued after 20 hours. Only in the severest cases of veronal poisoning do the pupils contract and become fixed as in acute morphine poisoning

Detection of Veronal

1. Veronal may be sublimed almost without decomposition when heated in a test-tube and well-formed crystals are obtained. The aqueous solution of these veronal crystals reacts faintly acid to litmus paper.

¹ W. Autenrieth. A Case of Medinal Poisoning and Detection of Veronal in the Urine. *Ber d. Deutsch. pharmaz Ges.* 31 (1921) Heft 3.

2. Veronal is soluble in solutions of potassium or sodium hydroxide, ammonia and sodium carbonate. If these solutions are as nearly saturated as possible, dilute hydrochloric acid will precipitate veronal unchanged

3. The melting-point of pure veronal is 187-188°. If the substance suspected of being veronal is mixed with the same quantity of pure veronal, the melting-point should not change.

4. With Millon's reagent, or a solution of 1 gram of yellow mercuric oxide in 2 cc. of nitric acid, as well as with Denigès reagent, an aqueous solution of veronal gives a white, gelatinous precipitate soluble in a large excess of Millon's reagent.

Detection of Veronal in Cadaveric Material

In examining cadaveric material (liver, spleen and kidneys) from a man, who had taken veronal, thinking it was a remedy for tapeworm (kamala), G. and H. Frerichs¹ isolated small quantities of the drug. Following the Stas-Otto process, they extracted the aqueous tartaric acid solution with ether and evaporated the ether extract. They recrystallized the residue from a little hot water, using animal charcoal to remove color, and identified the crystals obtained as veronal by the tests given above. In Chapter V of this book (page 567) the subject of veronal or medinal poisoning has received special treatment under the heading "Detection and Estimation of Veronal in Urine."

Apparently veronal, as Panzer² states, is not retained in the cadaver as such longer than 4 weeks. It may also happen in veronal poisoning that not a trace of the poison can be found in the cadaver. Such may be the case, for example, when death has not ensued for quite a long time after the last dose of veronal was taken and consequently all the poison has been eliminated through the kidneys.

In examining by the Stas-Otto process freshly removed organs of a woman, who had died under the suspicion of having been poisoned, Heiduschka³ found only very small quantities of veronal.

Quantity	Material	Veronal
100 cc.	Urine	0.0246 gram
200 grams	Parts of liver, lungs, spleen and heart	0.014 gram
200 grams	Parts of oesophagus, stomach and contents, large and small intestine, and bile	0.0079 gram

¹ G. and H. Frerichs. Detection of Veronal Poisoning. *Archiv d. Pharmaz.* 244 (1906), 86.

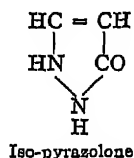
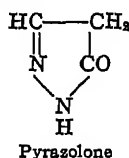
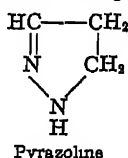
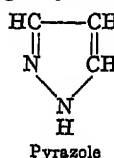
² Th. Panzer: Detection of Veronal. *Vierteljahrsschr. f. gerichtl. Medizin* (3) 36 (1908), 311.

³ A. Heiduschka: Forensic Detection of Veronal. *Archiv d. Pharmaz.* 249 (1911), 322.

It was to be expected that larger quantities of the hypnotic would be found in the urine but this did not turn out to be true in the case examined by Heiduschka. Apparently it was one of those cases, the possibility of which has been pointed out by Panzer, where death from veronal poisoning has not occurred until most of the drug has been eliminated from the body

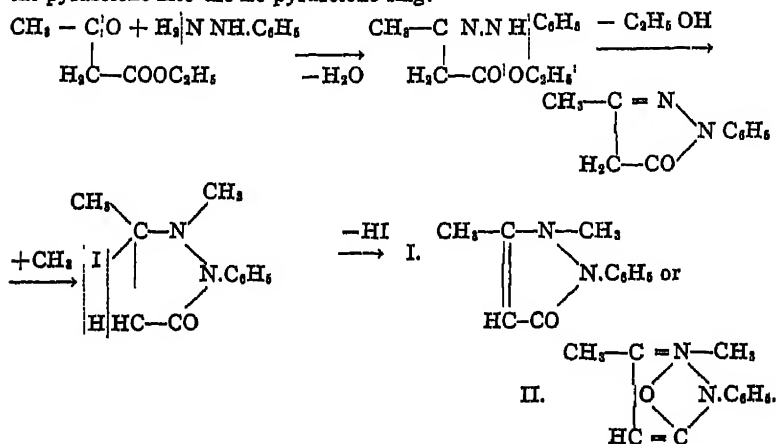
ANTIPYRINE

Antipyrine, according to its discoverer Knorr (1884), is to be regarded as a derivative of pyrazole, that is, of iso-pyrazolone. All derivatives of pyrazole contain a five-atom, heterocyclic ring consisting of 3 carbon and 2 nitrogen atoms:



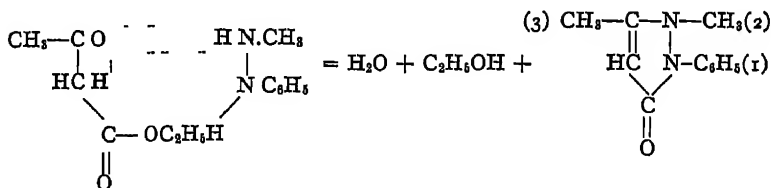
Iso-pyrazolone is tautomeric with pyrazolone and is known only in the form of alkyl and phenyl-derivatives.

Preparation.—I The oily phenyl-hydrazine-aceto-acetic ester, formed by mixing phenyl-hydrazine and aceto-acetic ester, is separated from the water of the reaction and warmed in the water-bath until a test-portion solidifies upon cooling. The phenyl-methyl-pyrazolone thus obtained is washed with ether and heated at 100–110° with methyl iodide in methyl alcohol solution. The methyl iodide addition-product formed is treated with sodium hydroxide solution. Antipyrine is formed with cleavage of hydriodic acid and rearrangement of the pyrazolone into the iso-pyrazolone ring:



I = Antipyrine-formula of Knorr; II = Antipyrine-formula of Michaelis.

II. Antipyrine is formed direct by heating aceto-acetic ester with symmetrical methylphenyl-hydrazine:



Crude antipyrine is purified by crystallization from ether, ligroin or toluene

Properties.—Antipyrine forms monoclinic, tabular crystals having a bitter taste and melting at 113° . One part of antipyrine is soluble in less than 1 part of cold water, in about 1 part of alcohol, 1 part of chloroform, and 50 parts of ether. An aqueous antipyrine solution has a neutral reaction to litmus, although this compound forms salts with acids that usually crystallize well.

Physiological Properties and Secondary Effects.—In doses of 1–2 grams antipyrine acts as an antipyretic, causing a drop in temperature within 4 hours frequently with copious sweating. Antipyrine is also valuable as a sedative and anodyne. According to Meyer and Gottlieb (*"Pharmakologie"* 1920), secondary effects rarely appear when the dose is not too large, that is, less than 20 grams. A very remarkable fact is the idiosyncrasy exhibited by many persons for antipyrine. The most frequent secondary effect is an antipyrine exanthema which is troublesome but not dangerous. More severe skin affections occur only in case of idiosyncrasy, such as inflammatory swelling of the skin of the face, also the skin of the genitals, and symptoms of severe irritation of mucous surfaces, that is, conjunctivitis, catarrh of the nose, throat and larynx. More frequently there is also nausea, retching and vomiting, rarely bloody vomitus, pains in the abdomen, and diarrhoea. The most frequent secondary effect connected with the urino-genital organs is a decrease in the secretion of urine; the appearance of albumin and sugar has also been observed in individual cases. Derangements of the respiratory organs appear in various forms: oppression and pains in the chest, painful respiration with a feeling of suffocation may occur in connection with symptoms of irritation in the nose and larynx, the appearance of which is probably attributable to the same anatomical changes, that is, swelling of the mucous surfaces of the respiratory passages (Lewin).

Detection of Antipyrine

1. **Ferric Chloride Test.**—Add 1–2 drops of dilute ferric chloride solution to an aqueous antipyrine solution. It will produce a deep red color that can be seen even in a dilution of 1:100,000. Dilute sulphuric acid changes the red to a pale yellow color.

2. **Tannic Acid Test.**—Tannic acid solution produces a white precipitate, when added to an aqueous antipyrine solution. Obviously this test is not characteristic of antipyrine.

3. **Fuming Nitric Acid Test.**—Add 1–2 drops of fuming nitric acid to an antipyrine solution. The solution will turn green. Then heat to boiling and add another drop of fuming nitric acid. The green color will change to red. This test is distinctly given by 1 cc. of an aqueous antipyrine solution (1.200).

4. **Nitroso-antipyrine Test.**—Add a few drops of potassium or sodium nitrite solution to an aqueous antipyrine solution and then dilute sulphuric acid. A green or blue color will appear. A few drops of acetic acid may be substituted for sulphuric acid but the solution must be heated. With concentrated antipyrine solutions green crystals of nitroso-antipyrine, $C_{11}H_{11}(NO)ON_2$, will separate after some time.

Detection of Antipyrine by the Stas-Otto Process

Ether extracts only small quantities of antipyrine from a solution containing much tartaric acid. Ether, or better chloroform, extracts by far the greater part of the antipyrine when the solution has been made alkaline with sodium hydroxide solution or ammonia. If the material contains notable quantities of antipyrine, usually it will appear in all ether and chloroform extracts in the Stas-Otto process. Antipyrine differs from most of the alkaloids by its solubility in water. To detect antipyrine, dissolve the residue from the ether or chloroform extract in a little water and examine this solution by the tests described above. Of the general alkaloidal reagents, picric acid and potassium bismuthous iodide are especially adapted for the precipitation of antipyrine. Antipyrine may be weighed in the form of the yellow crystals of antipyrine picrate, $C_{11}H_{12}ON_2.C_6H_2(NO_2)_3(OH)$.

Detection of Antipyrine in Urine

Antipyrine is easily absorbed and rapidly distributes itself in the human organism, so that it can be detected in all organs at once. Its elimination through the kidneys is proportionately rapid and it occurs in the urine partly unchanged and partly conjugated with sulphuric acid. Antipyrine-urine has a yellow to blood-red color and according to Penzoldt sometimes exhibits dichroism, red in transmitted and green in reflected light. In such a urine antipyrine usually can be detected direct with ferric chloride. To detect antipyrine with greater certainty, especially when the urine has a very dark color or contains

aceto-acetic acid, as is frequently the case in diabetes mellitus, render a considerable quantity of the urine strongly alkaline with ammonia or sodium hydroxide solution. Then extract with chloroform, pour this extract through a dry filter, and evaporate or distil off the solvent. Dissolve the residue in a little water and test the filtered solution for antipyrine with ferric chloride and with fuming nitric acid. Antipyrine is so easily absorbed that even an hour after it has been taken it will impart a reddish color to the urine and may be detected by the ferric chloride test. After about 25 hours the red color of the urine disappears but elimination after a dose of 2-3 grams of antipyrine continues for 36 hours. After a dose of 5 grams of antipyrine, no test is given at the end of about 50 hours. Jonescu¹ recommends a zone-test for urine by adding very dilute ferric chloride solution as an upper layer. If the urine contains antipyrine, a fine red ring will appear. In doubtful cases repeat the test with normal urine free from antipyrine. Moderate doses of antipyrine, 1-2 grams daily, apparently have no influence upon sulphur-metabolism, whereas larger doses, 5 grams and more, seem to diminish sulphate-sulphuric acid somewhat and to increase somewhat more ethereal sulphuric acid. Small doses of antipyrine are eliminated in the urine unchanged and only after larger doses does partial conjugation with sulphuric acid occur. In this manner the organism protects itself against larger doses by conjugation with a certain amount of the antipyrine, whereas the remainder passes off unchanged in the urine. To detect antipyrine itself in urine, Jonescu recommends precipitation by means of potassium bismuthous iodide solution suggested by Thoms. For this purpose acidify the urine with sulphuric acid, precipitate with the bismuth reagent, filter the precipitate by suction, and wash well with faintly acidulated water. Then triturate the precipitate with crystallized sodium carbonate and 10 per cent sodium hydroxide solution, and extract thoroughly with chloroform in a glass-stoppered separating funnel. Distil or evaporate the solvent. Antipyrine usually gives a crystalline residue. The purine-bodies of normal urine are also precipitated by the bismuth reagent but they do not give a residue with chloroform. Gadamer recommends silver carbonate for the decomposition of the bismuth precipitate.

Quantitative Estimation of Antipyrine as Picrate

(a) **Gravimetric Estimation.**—According to Riedel,² antipyrine (Mol. Wt. 188) may be weighed as the picrate, $C_{11}H_{13}ON_3 \cdot C_6H_3(NO_2)_3OH$ (Mol. Wt. 417). For this purpose, dissolve 0.5 gram of antipyrine, or an antipyrine mixture corresponding approximately to this quantity, in 50 cc. of water. Add 5-6 cc. of n-hydrochloric acid, heat to boiling, and add 10 cc. of a cold saturated alcoholic solution of picric acid. Allow to stand in the cold for several hours (preferably until the following day) and collect the precipitated antipyrine picrate crystals in a weighed Gooch crucible, adding by rinsing those crystals that adhere to the sides of the beaker used for precipitation. Remove completely all liquid by suction and dry crucible and precipitate at about 95° to constant weight in a steam drying-closet. Washing the crystals with water, alcohol, etc.,

¹ D. Jonescu: Elimination of Antipyrine from the Human Organism. Ber. d. Deutsch pharm. Ges. 16 (1906), 133.

² J. D. Riedel: Riedel's Berichte 1907, page 67.

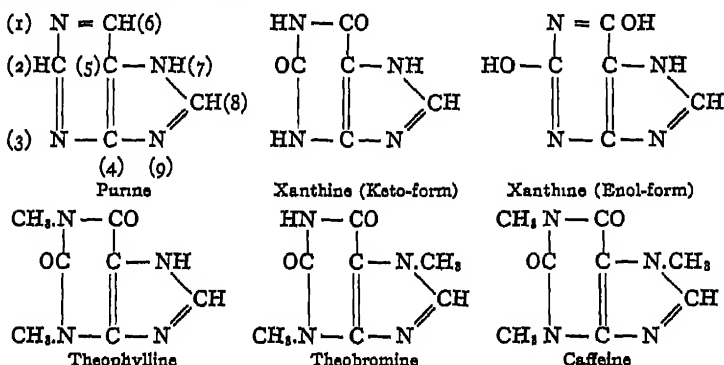
is not permissible on account of their solubility. Since the quotient Antipyrine · Picrate = $188.417 = 0.451$, the quantity of antipyrine is obtained by multiplying the weight of precipitate by 0.451 . As a check, the melting-point of the crystals should be determined. Pure antipyrine picrate melts at 187° .

(b) **Volumetric Estimation.**—Lémaire¹ precipitates antipyrine in aqueous solution with excess of 0.05 *n*-picric acid solution and measures in an aliquot part of the filtrate from the antipyrine picrate precipitate excess of picric acid with 0.1 *n*-sodium hydroxide solution, using phenol-phthalein as indicator. A liter of 0.05 *n*-picric acid solution contains 0.05 $C_6H_3(NO_2)_3(OH) = 229.20 = 11.45$ grams of pure picric acid. This quantity of picric acid will precipitate 0.05 $C_{11}H_{12}ON_2$ grams = $188.20 = 9.4$ grams of antipyrine.

Procedure.—Put 10 cc of an approximately 5 per cent aqueous antipyrine solution in a 100 cc graduated flask, fill to the mark with 0.05 *n*-picric acid solution, that is, with 90 cc, shake well, allow to stand in the cold for 0.5 – 1 hour, and filter 50 cc through a dry paper into a dry graduated flask. Now add 1 cc of phenol-phthalein solution and titrate with 0.1 *n*-sodium hydroxide solution to a permanent red color. If the number of cc. of 0.1 *n*-alkali used is quadrupled and 90 is subtracted from the number obtained, it will give the number of cc of 0.05 *n*-picric acid combined with the antipyrine in the 10 cc taken.

CAFFEINE

Caffeine together with theophylline and theobromine constitute a group of closely related substances derived from xanthine by substituting methyl for two or three atoms of hydrogen. Caffeine is $1, 3, 7$ -trimethyl-xanthine, theophylline, $1, 3$ -dimethyl-xanthine, and theobromine, $3, 7$ -dimethyl-xanthine. All these substances are derivatives of purine. Their close relation to purine is shown by their structural formulae:



Properties.—Caffeine, also called theine, crystallizes in white, shining needles. It is soluble in 80 parts of water, giving a colorless solution with a neutral reac-

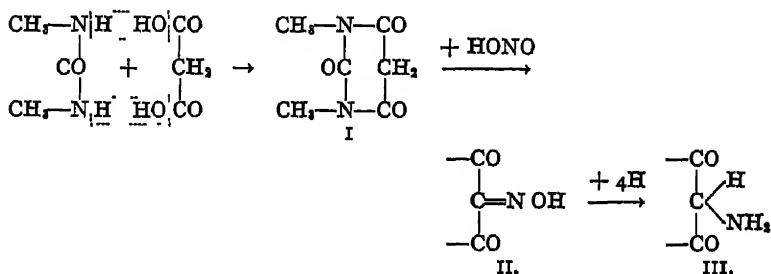
¹ P. Lémaire: Répertoire de Pharmacie 1904, No 11.

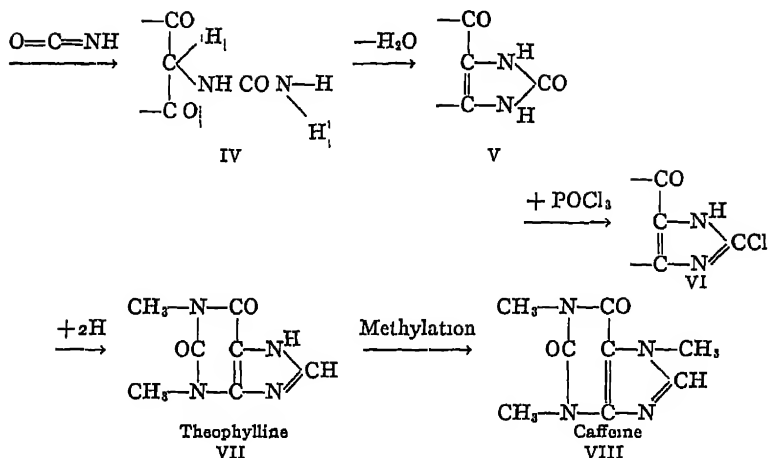
tion and a bitter taste. It is quite easily soluble in hot water (1:2). It requires for solution about 50 parts of alcohol, 9 parts of chloroform, but is only slightly soluble in ether, absolute alcohol, benzene and petroleum ether. In crystallizing from hot water, caffeine combines with 1 molecule of water, a part of which it loses upon exposure to air and all when dried at 100°. It melts at 230°, but somewhat above 100° begins to volatilize in small quantity and at 180° to sublime in colorless needles without leaving a residue. Concentrated sulphuric and nitric acid dissolve caffeine without color. Caffeine is a very weak base and its salts are decomposed by water. Therefore, caffeine can be extracted at least partially by ether, or better by chloroform, from an aqueous tartaric acid solution.

Synthetic Caffeine.—The synthesis of caffeine was first accomplished by heating the silver salt of theobromine for a long time at 100° with methyl iodide. This reaction proceeds more smoothly, if theobromine (1 Mol) and potassium hydroxide (1 Mol) in alcoholic solution are heated with methyl iodide (1 Mol) in a closed vessel for 6 hours at 100° (E. Schmidt), or if the solution of theobromine in excess of sodium hydroxide solution is shaken with dimethyl sulphate, $(\text{CH}_3\text{O})_2\text{SO}_2$. In the same manner theophylline and paraxanthine, or 1,7-dimethyl-xanthine, may be converted into caffeine by methylation. Quite a remarkable synthesis of caffeine was effected by Emil Fischer. Starting with α , β -dimethyl-urea, obtained from methyl cyanate and methyl amine:



he condensed it with malonic acid to dimethyl-malonyl urea, or dimethyl-barbituric acid (I). This compound was converted by nitrous acid into dimethyl-nitroso-barbituric acid (II). The latter upon treatment with nascent hydrogen, that is, by the action of hydriodic acid, gave dimethyl-uramil (III). Since this compound is a primary amine, it adds cyanic acid in the form of its potassium salt, yielding dimethyl-pseudo-uric acid (IV). Elimination of water and ring-closure takes place, when the free acid is heated with anhydrous oxalic acid, and dimethyl-uric acid (V) is formed. Treatment of the latter with phosphorus oxychloride (POCl_3) converts it into chlorotheophylline (VI). Reduction by means of hydriodic acid substitutes hydrogen for chlorine and gives theophylline (VII). This synthetic 1,3-dimethyl-xanthine has been shown to be identical in every respect with natural theophylline occurring in tea. By methylation theophylline is converted into caffeine (VIII):





Physiological Action.—In small doses caffeine acts as a stimulant upon the central nervous system so that the efficiency of the brain and spinal cord is increased. The agreeable effects produced by drugs containing caffeine are due to this property. Their irritability is increased by larger doses of caffeine to such an extent that it amounts to tetanus. Aside from that, caffeine gives rise to a muscular change, so that the striped muscles become as rigid as in death. Caffeine acts as a powerful diuretic. Inasmuch as diuresis is also excited even in the excised kidneys, it cannot be dependent, at least not exclusively, upon changes in blood-pressure.

The symptoms of intoxication, appearing in acute poisoning by caffeine, are like those resulting from the state of excitation occasioned by drunkenness: dizziness, ringing in the ears, headache, trembling, uneasiness, sleeplessness, confusion of ideas, delirium, palpitation of the heart, albuminuria, stiffness of the muscles, and even real convulsions. Death ensues as a result of paralysis of the heart. Kobert cites the case of a man who had taken about 4 grams of caffeine. After 15 minutes there occurred burning in the chest, trembling, dizziness, emesis and diarrhoea, marked diuresis, abdominal pains, highly accelerated pulse and collapse. After 9 hours collapse subsided and 3 days later the patient was again quite normal. But even after a dose of 0.5–0.6 gram of caffeine, a condition of excitation like that of drunkenness, sleeplessness, dizziness, muscular tremors, inclination to emesis and diarrhoea, as well as severe micturition may also appear. After larger doses of about 1 gram, in addition to these symptoms, palpitation of the heart appears, considerable increase in the frequency of the pulse and irregularity in the heart's action. Without severer secondary effects, caffeine poisoning is accustomed to disappear. (Hans Meyer-Gottlieb.)

Elimination of Caffeine.—Only a very small part of the caffeine, and then only when larger quantities of it have been taken, passes through the organism unchanged and appears as such in the urine. Another part of the caffeine, about 10 per cent of that taken, undergoes demethylation in metabolism, since

mono- and dimethyl-xanthine appear in the urine. The remainder of the caffeine must undergo conversion into the normal end-products of human metabolism.

Detection of Caffeine

Ether will extract more caffeine from an aqueous alkaline solution than from an aqueous tartaric acid solution. Since caffeine dissolves with some difficulty in ether, but more easily in chloroform, the latter solvent is usually employed after the solution has been made alkaline with ammonia. After distillation of solvent, caffeine appears in concentric clusters of long, shining needles. In an analysis by the Stas-Otto method, caffeine will appear in all three extracts. Chloroform, benzene and also amyl alcohol extract caffeine completely from acid solution, whereas petroleum ether will extract it neither from acid nor alkaline solution. Caffeine in dilute sulphuric acid solution (1:1000) is precipitated only by such general alkaloidal reagents as phospho-molybdic acid, phospho-tungstic acid, and potassium bismuthous iodide.

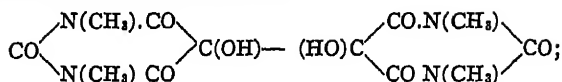
1. Amalic Acid Test.—Gradually evaporate caffeine to dryness in a small dish upon the water-bath with a few cc. of strong chlorine water,¹ taking about 10 times the quantity of the latter. A red to red-brown residue will remain. If a very little ammonium hydroxide solution is at once added, a fine purple-violet color will appear. This test may be made by covering the dish containing the residue with a glass plate moistened with a drop of strong ammonia. Or two matched watch-glasses may be used, the material containing caffeine being evaporated to dryness with chlorine water upon one glass which is then placed for a short time upon the other glass containing a drop of strong ammonia.

This test is not confined to caffeine, for it is also given by theophylline and theobromine. They, however, require rapid evaporation with about a hundred times the quantity of chlorine water. The residue left upon evaporation is also red but the shades of color that appear with ammonia are somewhat different. But caffeine may be separated from theophylline and theobromine by the fact that benzene will extract it from alkaline solution and leave the other two bases behind. If the solution is then acidified, chloroform will extract theophylline and theobromine. According to Gadamer, theophylline further differs from caffeine and theobromine in giving a red color with the diazonium reagent.²

¹ A convenient method of preparing saturated, aqueous chlorine solution is to heat potassium chlorate with hydrochloric acid and pass the chlorine into a small quantity of water.

² See "Preparation of Reagents" page 641.

Amalic acid is tetramethyl-alloxantine.



and so is formed from 2 molecules of caffeine or theophylline with cleavage of the remainder of the molecule, that is, $-\text{N}(\text{CH}_3) \text{CH N}-$ or $-\text{NH}.\text{CH N}-$. Amalic acid forms colorless crystals that easily redden in the air, are difficultly soluble in water and insoluble in absolute alcohol. In contact with ammonia, amalic acid takes on a purple-red color and a blue color when moistened with potassium or sodium hydroxide solution.

2. Tannic Acid Test.—This reagent, added to an aqueous caffeine solution, causes a heavy white precipitate soluble in an excess of the acid. This test is not characteristic of caffeine.

B. Examination of Ether Extract of Aqueous Alkaline Solution

This ether extract may contain alkaloids and other basic substances. The exceptions are the phenolic bases, morphine, apomorphine and narceine

The aqueous tartaric acid solution withdrawn in a separating funnel from the last ether extract should be made strongly alkaline with sodium hydroxide solution ¹ The alkali will liberate alkaloids from their salts and combine with the phenolic bases. Then thoroughly extract this solution at once with about the same volume of ether. Separate the ether from the aqueous solution and again extract with a fresh quantity of ether. Withdraw the latter also. In certain cases 3-4 such extractions may be required. This is necessary when a substance like strychnine, that is difficultly soluble in ether, is under examination. Mix the combined ether extracts in a dry flask, stopper loosely and set aside for 2-3 hours. Carefully decant the ether through a dry paper and evaporate (**Extinguish all flames!**) in a small glass dish upon a water-bath that has previously been warmed

¹ When the examination is concerned exclusively with one of the "ester-alkaloids," such as atropine, hyoscyamine, cocaine, scopolamine, or with physostigmine, or with the lactone, pilocarpine, but not with morphine or apomorphine, it is better to render the solution alkaline with sodium carbonate, or better bicarbonate, rather than with sodium hydroxide solution. They will set the alkaloids free from their salts without causing saponification or cleavage after that has taken place:



Larger quantities of ether should first be distilled to a volume of 10-15 cc. and this more concentrated ether solution then evaporated in a glass dish as described above. Special precaution should be taken not to allow even a drop of the aqueous solution, that usually separates in small quantity when the ether extract is allowed to stand in a flask, to mix with the filtered ether solution. Moreover the residue from the ether solution should not be allowed to stand for a needlessly long time upon the warm water-bath, otherwise alkaloids that may be present have a tendency to become viscous and conium and nicotine may volatilize.

The residue from the ether extract of the aqueous alkaline solution may contain nearly all the alkaloids with the exception of the phenolic bases, morphine, apomorphine and narceine, as well as other organic substances that are basic in character. It should be examined for

Coniine	Atropine	Hydrastine
Nicotine	Scopolamine	Pilocarpine
Aniline	Cocaine	Quinine
Veratrine	Physostigmine	Caffeine
Strychnine	Codeine	Antipyrine
Brucine	Narcotine	Pyramidone

First, examine the appearance of the residue both macroscopically and microscopically. Taste it cautiously. Certain alkaloids may be recognized beforehand by this test. Tests for any such substances in the residue should be made first.

The various alkaloids appear in the residue as follows:

Strychnine.—Very fine needles having an exceedingly bitter taste.

Brucine.—White, amorphous powder having a very bitter taste.

Veratrine.—Amorphous powder having a sharp, burning taste.

Atropine, Quinine and Codeine.—Varnishes that are usually resinous and sticky but rarely crystalline.

Caffeine.—Long, frequently concentrically arranged needles having a faintly bitter taste.

Pyramidone.—Fine, crystalline needles easily soluble in water and having a faintly bitter taste.

Scrape the residue together with a platinum or nickel spatula, or with the clean blade of a pocket-knife, to get some idea as to the quantity of alkaloid present.¹

¹ The author evaporates ether and chloroform extracts upon accurately weighed watch-glasses, removing the last 5-6 cc. of solvent in the vacuum apparatus shown upon pages 96 and 111.

Preliminary Tests for Nitrogenous Bases

Dissolve a test-portion of this residue in 2-3 cc. of water, adding a drop of dilute hydrochloric acid. Filter this solution, if necessary, distribute it in several small test-tubes, and add to each portion one of the general alkaloidal reagents, such as mercuric chloride, potassium mercuric iodide, potassium bismuthous iodide, picric acid, phospho-molybdic acid and phosphotungstic acid. If an alkaloid is present, abundant precipitates will appear in these tests. If characteristic precipitates are not obtained, no alkaloid is present.

In making special tests for alkaloids, distribute all the residue upon several small watch-glasses and test each by a reaction more or less specific for a particular alkaloid. If a definite substance is indicated, confirm the first test by other reactions. The "Synopsis of Group II B" (see page 284) is useful in this connection for reference, and also the comparison of the behavior of known alkaloids toward concentrated sulphuric and nitric acid (reagents of Froehde, Marquis, Mandelin and Mecke given on page 237).

An alternative procedure is the following. Dissolve the residue in a little hot alcohol, filter the solution, distribute upon watch-glasses, and evaporate at a gentle heat. The residues may then be tested. Mauch¹ dissolves the residue in 75 per cent. aqueous chloral hydrate solution and uses this solution in testing for alkaloids. The details of this method are given in Chapter V of this book (see page 572).

Purification of the Ether Residue

When the ether residue is contaminated with greasy, resinous, colored or fatty substances, as is often the case in the examination of cadaveric material, many of the tests will either fail entirely or give uncertain results. In this case the residue should be purified in one of two ways.

1. Thoroughly mix the residue with cold water containing hydrochloric acid. Filter to remove insoluble matter (fatty or resinous substances), add sodium hydroxide solution to the filtrate until alkaline and extract with ether. The alkaloids obtained by evaporating the solvent are usually quite pure.

2. This method consists in dissolving the residue in hot amyl alcohol. Extract this solution with a few cc. of very dilute sulphuric

¹ R. Mauch. Contribution at the Congress of the Association of German Druggists, Strassburg 1907.

acid and withdraw the acid solution from the separating funnel. Amyl alcohol will retain greasy and colored impurities, and the alkaloids will be in the aqueous solution as sulphates. Add sodium hydroxide solution in excess and extract with ether. This method of purifying the alkaloidal residue is especially recommended, when considerable coloring matter is present.

Physiological Test.—If the ether extract upon evaporation leaves only a very slight residue, and one that does not taste bitter, frequently no alkaloid is present. The residue consists of fat, resinous matter, or even of traces of some nitrogenous substances, such as peptones (peptide-like bodies) or other cleavage-products of proteins. The examination of cadaveric material by the Stas-Otto process for alkaloids, even after the most painstaking work, frequently produces ether extracts that yield a slight residue when no alkaloid is present. To make perfectly sure as to the presence or absence of alkaloid, dissolve most of the residue in a little water containing hydrochloric acid and test this solution with general alkaloidal reagents. Only when these tests give characteristic precipitates, should the ether residue be examined chemically or physiologically for alkaloid.

In an investigation of more importance, to exclude all possibility of overlooking traces of a particular poison, dissolve a portion of the ether residue in very dilute hydrochloric acid (about 0.5 per cent HCl). Evaporate this solution upon the water-bath, dissolve the dry residue in 1–2 cc. of water, and inject this solution with a hypodermic syringe into the lymph-sac of a lively frog. Triturate a second portion of the ether residue with a small quantity of flour, or dry bread, and a little sugar,¹ and feed this mixture to a white mouse. If in the course of several hours the experimental animal exhibits no symptoms of poisoning, such as uneasiness, trembling, convulsions, tetanus, diarrhoea, it is quite probable that none of the more powerful alkaloids is present. If a special test has to be made for an alkaloid having mydriatic or myotic properties, the solution of the ether residue prepared as described above should be introduced into one eye of a cat to determine whether or not it causes expansion or contraction of the pupil.

Putrefactive Products of Human Organs and the Stas-Otto Method

Panzer² has thoroughly investigated the products that arise as a result of putrefaction of different parts of the human body, particularly with regard to their behavior toward general alkaloidal reagents. The object of this study was to determine whether, in the ordinary procedure of forensic analysis by the Stas-Otto method, ptomaines also may not pass into ether and chloroform extracts, thus making it difficult to detect alkaloids or leading to false conclusions as to the presence of the latter. According to the results of these investigations, the brain alone of all the human organs examined yields substances that give the general reactions of alkaloids even after further purification. Consequently in experiments of this character it is better to exclude the brain entirely, if this

¹ Sugar alone fed to mice easily occasions diarrhoea.

² Th. Panzer: Contribution to the Knowledge of Putrefaction of Human Organs. A Contribution to the Stas-Otto Method. *Zeitschr. f. analyt. Chem.* 47 (1908), 572.

is possible. When the ether extract of the aqueous solution, rendered alkaline with sodium hydroxide, contains amorphous substances that give precipitates with general alkaloidal reagents, these should be removed by dissolving in hydrochloric acid and extracting with ether. This solvent will extract these amorphous substances but not alkaloids. In addition to basic products of putrefaction, Panzer has also discovered acid substances, such as hydro-para-cumic acid.

CONINE

Coniine, or α -normal-propyl-piperidine, $C_8H_{16}.NH$, occurs in all parts of spotted hemlock (*Conium maculatum*) as the dextro-rotatory form, presumably combined with lactic acid. Associated with it are n-methyl-coniine, γ -coniceine and pseudo-conhydrine. It occurs most abundantly (0.2–0.9 per cent) in not entirely ripe fruit of plants about 2 years old. Leaves and flowers are poorer in alkaloids.

Properties.—Naturally occurring dextro-coniine is a colorless, oily liquid that gradually turns yellow and brown in the air and in more dilute condition has a stupefying odor suggestive of mouse urine. In an atmosphere free from oxygen it boils without decomposition at 166° . Its angle of rotation is $[\alpha]_D = +15.7^\circ$ at 19° . In spite of its high boiling-point, coniine volatilizes in considerable quantity even at ordinary temperature. Coniine is rather difficultly soluble in water at 15° (1:909) but even less soluble in hot water. For this reason a cold, saturated, aqueous coniine solution becomes milky when warmed.

Constitution and Synthesis.—Coniine is a secondary base as shown by its behavior. Heated with acetic anhydride, it forms N-acetyl-coniine.



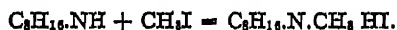
and when shaken with benzoyl chloride and sodium hydroxide solution, it gives N-benzoyl-coniine:



and with nitrous acid nitroso-coniine:

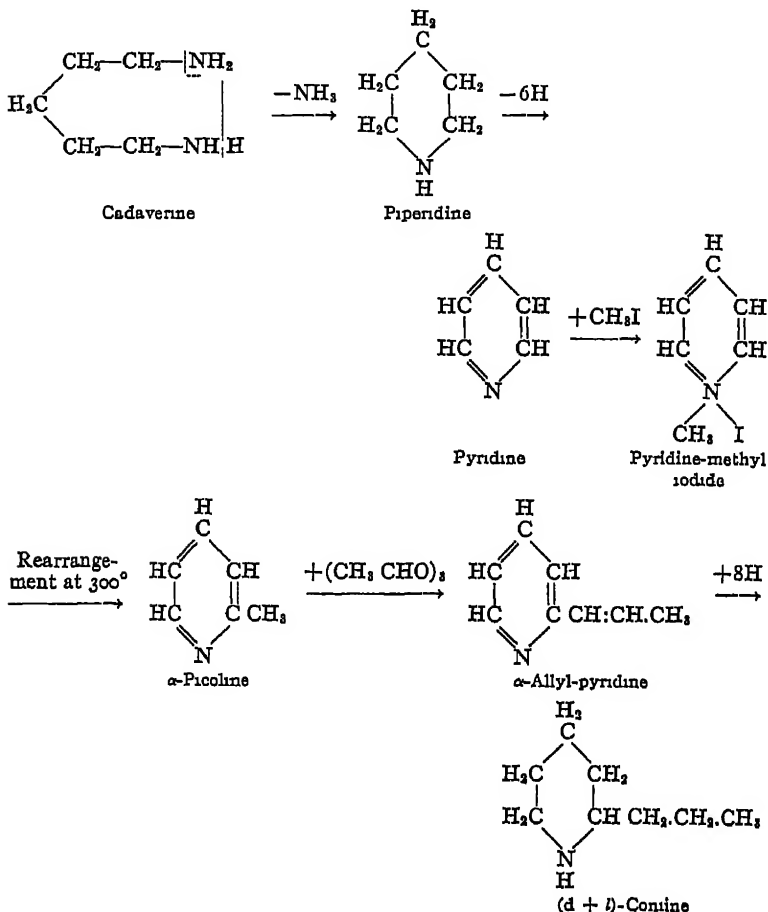


Finally methyl iodide and ethyl iodide convert coniine into the iodide of methyl and ethyl-coniine:



Coniine may be prepared synthetically by means of the same cadaveric alkaloid, or ptomaine, that is formed in largest quantity in the putrefaction of cadavers, that is, cadaverine or penta-methylene-diamine. If the hydrochloride of this base is heated by itself, piperidine is formed as a result of loss of ammonia and ring-closure. Piperidine, oxidized with silver oxide or heated with concentrated sulphuric acid, loses six atoms of hydrogen and is converted into pyridine. The latter as a tertiary base adds methyl iodide and forms pyridine-methyl iodide which at 300° undergoes rearrangement to the isomeric α -picoline hydriodide. Free picoline, obtained by means of potassium hydroxide, condenses when heated

with paraldehyde with loss of water forming α -allylpyridine. Upon reduction with sodium and alcohol, this compound is converted into inactive conune. If the salt formed by inactive conune with dextro-tartaric acid is crystallized from water, dextro-conune tartrate crystallizes out first. When the latter is treated with sodium hydroxide solution, it gives dextro-conune which is identical with natural dextro-conune.



Toxic Action.—As a rather strong base, conine acts locally as a caustic and is therefore easily absorbed even upon external application. Then it exerts a central and peripheral action. The symptoms of intoxication consist of burning in the mouth, itching in the throat, flow of saliva, heaviness in the head, dizziness, nausea, inclination to emesis, tormenting thirst, drowsiness, indistinct vision and hearing, enlarged pupils, weakness in the extremities, great infirmity and cramp of the sural muscles. The principal symptoms, however, consist of

paralysis first of the lower extremities, as a result of which there may be unsteadiness of gait or entire lack of support, then of paralysis of the arms and muscles of respiration. Death ensues from paralysis of respiration. Coniine that is absorbed is eliminated by the lungs and urine. The materials that should be examined are vomitus and stomach contents, blood and organs rich in blood, liver, spleen, lungs, as well as kidneys, and urine. Coniine is said to be fairly resistant to cadaveric putrefaction.

The subject of cadaveric coniine is considered in connection with ptomaines (see page 463).

Detection of Coniine

General alkaloidal reagents especially sensitive to coniine are iodo-potassium iodide (1:8000), phospho-molybdic acid (1:5000), potassium mercuric iodide (1:8000), and potassium bismuthous iodide (1:5000). Coniine picrolonate forms yellow rhombohedrons, melting at 195.5°, and easily soluble in alcohol and warm ether. Gold and platinum chloride fail to precipitate coniine when the concentration is less than 1:100, whereas they precipitate nicotine when the concentration is as low as 1:5000. When coniine is present, the residue from the ether solution has the characteristic odor of this alkaloid. The following tests may be used for its further identification:

1. **Solubility Test.**—Dissolve a drop of coniine in sufficient water to give a clear solution. Gently heat the solution and it will become milky from separation of coniine. A coniine solution that is milky when hot will become clear on cooling. Aqueous coniine solutions have an alkaline reaction. Test the solution with red litmus paper.

2. **Crystallization Test.**—Put a little coniine upon a watch-glass, or glass slide, and add 1-2 drops of hydrochloric acid. Evaporate to dryness and coniine hydrochloride will remain. Immediately after evaporation examine this residue with a microscope magnifying about 200 times. The colorless or faintly yellow crystals are needle-like, or columnar, and frequently are grouped in star-shaped clusters. They show the play of color characteristic of doubly refractive substances.

3. **Melzer's¹ Test.**—Add 2 drops of carbon disulphide and about 2 cc. of alcohol to a trace of coniine and allow it to stand for a few minutes. If 2-3 drops of an aqueous copper sulphate solution (1:2000) are added to this mixture, a distinct yellow to brown color will appear. A similar brownish color is produced by 1-2 drops of very dilute ferric chloride solution even in a coniine solution diluted

¹H. Melzer: Contributions to Forensic Chemistry. *Zeitschr. f. analyt. Chemie* 37 (1898), 345.

1:10,000. Dilute beforehand 1 gram of ordinary ferric chloride solution with water to 100 cc. Excess of copper sulphate and ferric chloride solution should be avoided. This reaction is not characteristic of coniine but merely shows the presence of a secondary base. Ether will extract the coloring-matter from coniine solutions rendered brown by copper sulphate, even after dilution with water 1:10,000. Nicotine does not give these colorations.

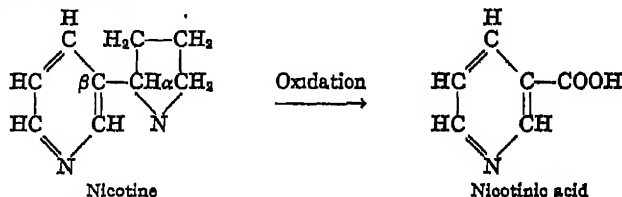
4. **Physiological Test.**—Subcutaneous injection of at least 15 milligrams of coniine hydrochloride will produce in a frog typical paralysis of the extremities (curare action). Nicotine does not exhibit this action, nor do the cadaveric coniines thus far known.

NICOTINE

Nicotine, $C_{10}H_{14}N_2$, combined with malic and citric acid, occurs in varying quantities (0.5–1.5 per cent.) together with other alkaloids in different species of *Nicotiana*.

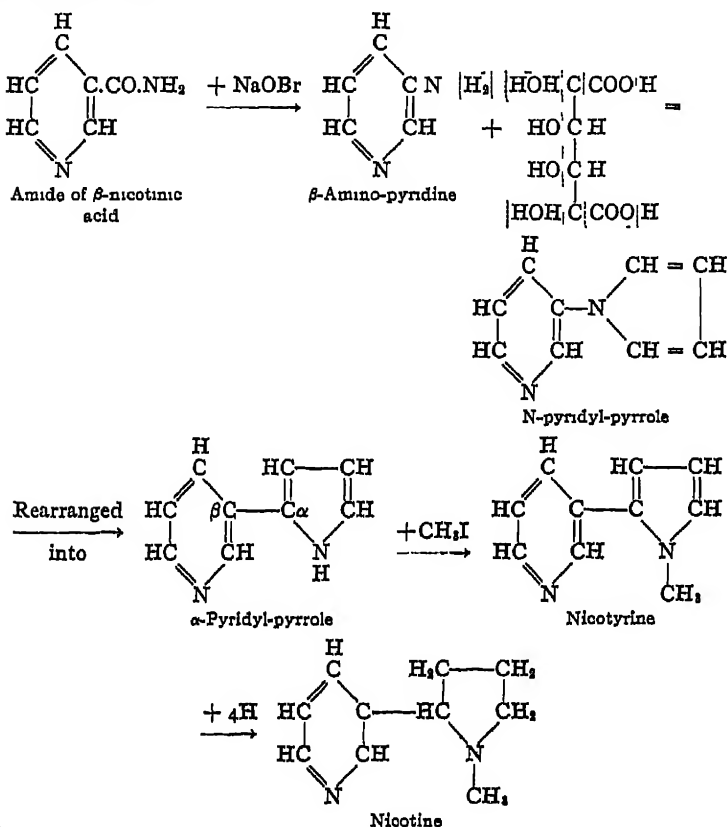
Properties.—Nicotine is a colorless liquid, soon turning yellow and brown upon exposure to air and in time becoming entirely resinous. It has a faint odor of tobacco and a sharp, burning and very persistent taste. In a current of hydrogen it boils without decomposition at 240 – 242° but during distillation in contact with air undergoes partial decomposition. The free alkaloid is strongly laevo-rotatory, $[\alpha]_D = -166^\circ$; but its salts are dextro-rotatory. Nicotine is miscible with water in all proportions (distinction from coniine) and freely soluble in alcohol, ether, benzene and petroleum ether. Aqueous and dilute alcoholic nicotine solutions react strongly alkaline to litmus but not to phenol-phthalein.

Constitution and Synthesis.—Nicotine is a di-acid base, since it forms well-crystallized salts with one and two equivalents of acid. It combines with 2 molecules of an alkyl iodide, for example, with $2\text{CH}_3\text{I}$, forming a di-iodo-methylate, $C_{10}H_{14}N_2 \cdot 2\text{CH}_3\text{I}$, from which moist silver oxide separates nicotine alkyl-hydroxide. By this behavior nicotine shows itself to be a di-tertiary base. Oxidized with nitric acid, chromic acid or potassium permanganate, nicotine is converted into nicotinic acid, or β -carboxy-pyridine. This shows that nicotine is a pyridine derivative having a side-chain in the β -position with respect to the pyridine nitrogen. This behavior is in accord with the structural formula proposed for nicotine by Pinner and substantiated several years later by Pictet's synthesis of nicotine.



Pictet, starting from nicotinic acid, effected the synthesis of nicotine. The amide of nicotinic acid obtained in the usual manner was converted, upon treat-

ment with sodium hypobromite according to the method of A W Hofmann, into β -amino-pyridine. This compound subjected to dry distillation with mucic acid forms a pyrrole-ring with loss of carbon dioxide and water, giving N-pyridyl-pyrrole. If the vapor of the latter is passed through a gently ignited tube, molecular rearrangement takes place with formation of the isomeric α -pyridyl-pyrrole which forms salt-like compounds with alkali metals. Methyl iodide acting upon the potassium salt forms nicotyrine which differs from nicotine in containing 4 atoms less of hydrogen. For the purpose of rendering the pyrrole-ring of nicotyrine more easily reducible for conversion into nicotine, nicotyrine was converted into a mono-substitution product by means of iodine in alkaline solution. This compound was then reduced to dehydro-nicotyrine by tin and hydrochloric acid. The dibromo-addition product of this compound upon reduction with tin and hydrochloric acid gave tetra-hydro-nicotyrine, or inactive nicotine. This racemic compound converted into its salt with dextro-tartaric acid was resolved into laevo-nicotine, identical with the natural base, and its optical opposite. According to this synthesis, natural nicotine is α -pyridyl-methyl-pyrrolidine:



Toxic Action.—Nicotine is one of the most violent poisons and is hardly inferior to hydrocyanic acid in toxic power and rapidity of action. It probably acts as a poison upon all classes of animals. Absorption of nicotine takes place even in a few seconds from the tongue, the rectum and the eye, somewhat more slowly from the stomach. It is also absorbed from the outer skin. In consequence of this rapidity of absorption, nicotine exhibits its toxic action very quickly, even after a few minutes. Elimination proceeds through the lungs and kidneys. Although not really corrosive in its action, nicotine in more concentrated form, especially as the free alkaloid, acts as a local irritant. After administration of fatal doses by the mouth, usually inflammation of the mucous lining of the stomach does not occur, for the action of nicotine is too rapidly fatal. Moreover, following brief stimulation, nicotine exerts a central paralyzing action upon the brain and spinal cord, and finally as a result of absorption acts upon different organs, such as the heart, eyes, and intestinal tract. In acute nicotine poisoning death ensues from paralysis of the respiratory center, but probably action upon the heart always takes place, although this usually does not lead to death.

The symptoms of nicotine poisoning have been accurately determined by various experimenters who have taken from 1 to 4 milligrams of this alkaloid. There is burning in the mouth and throat, increased secretion of saliva, headache, stupefaction, dizziness, indistinctness of vision and hearing, photophobia, parched throat, coldness of the extremities, emesis and enforced evacuation of the bowels. Respiration was accelerated and labored. At first the pulse increased in frequency but later changed, becoming at irregular intervals more and less frequent. After 45 minutes the experimenters swooned and in one case for 2 hours there were uninterrupted clonic convulsions, especially of the muscles of respiration, with trembling of the extremities and shaking of the whole body. In severer cases the gastric symptoms predominate. The intense diarrhoea that appears may be bloody, resulting in death in a few hours. On the other hand, in severe cases, for example, from tobacco enemas or from nicotine itself, convulsions, delirium and paralysis predominate and may prove fatal in 10-30 minutes.

In suspected nicotine poisoning, the material that should be selected for chemical examination is: urine, blood, stomach and intestines with contents, liver and lungs.

Detection of Nicotine

Ether or low-boiling petroleum ether will extract nicotine from an aqueous alkaline solution. Evaporation of the solvent leaves the alkaloid as an oily liquid having the odor of tobacco and a strong alkaline reaction. Most of the general alkaloidal reagents will precipitate nicotine from quite dilute solutions, in which respect this alkaloid is very different from coniine. Phospho-molybdic acid and potassium bismuthous iodide precipitate nicotine even in a dilution of 1:40,000; potassium mercury iodide in 1:15,000; gold chloride in 1:10,000, and platinum chloride in 1:5,000. Nicotine

picrolonate forms prismatic needles frequently arranged in clusters and melting at 213° . The following special tests serve for the identification of nicotine:

1. **Crystallization Test.**—Evaporate nicotine dissolved in dilute hydrochloric acid upon a watch-glass. A yellow varnish will remain. Microscopic examination will show it to be entirely amorphous. If kept for a long time in a desiccator over sulphuric acid, it will become indistinctly crystalline. This test is not of as much use in identifying nicotine as in differentiating it from coniine, the hydrochloride of which is characterized by its great tendency to crystallize.

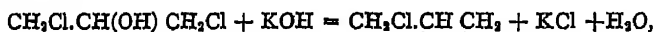
2. **Roussin's Test.**—Dissolve a trace of nicotine in ether, using a dry test-tube. Add to this solution about the same volume of ether containing iodine. Stopper and set the test-tube aside. The mixture will become turbid and deposit a brownish red resin, gradually becoming crystalline. After some time, ruby-red needles having a dark blue reflex will crystallize. These are "Roussin's crystals." If nicotine is old or resinous, as a rule it will not give these crystals.

3. **Melzer's¹ Test.**—If the alcoholic solution of a drop of nicotine is heated with about 2 cc. of epichlorohydrine,² the mixture after more or less time, depending upon the concentration, will become distinctly red. The limit of delicacy is 0.25 mg of nicotine. Under the same conditions coniine gives no color.

4. **Schindelmeyer's³ Test.**—If 5-10 mg. of non-resinous nicotine is treated first with a drop of formaldehyde solution (about 30 per cent.) free from formic acid and then with a drop of concentrated nitric acid, the mixture takes on a rose-red color. If nicotine and formaldehyde are in contact for several hours, the solid residue

¹ H. Melzer: Contributions to Forensic Chemistry. *Zeitschr. f. analyt. Chemie* 37 (1898), 345; Reactions of Coniine and Nicotine. *Zeitschr. d. allgem. österr. Apotheker-Vereins* 54 (1902), 65.

² Epichlorohydrine, obtained by the action of potassium hydroxide (1 Mol.) upon α -di-chlorohydrine, $\text{CH}_2\text{Cl}.\text{CH}(\text{OH}).\text{CH}_2\text{Cl}$, or β -dichlorohydrine, $\text{CH}_2\text{Cl}.\text{CHCl}.\text{CH}_2\text{OH}$:



is a colorless liquid insoluble in water and freely soluble in alcohol and ether. It has an odor like chloroform and a burning, sweetish taste.

³ J. Schindelmeyer: Detection of Nicotine. *Pharm. Zentralhalle* 40 (1899), 703.

treated with a drop of concentrated nitric acid gives an even finer color reaction, that is, a rose to a dark red color.

Only a little formaldehyde should be used, otherwise the solution becomes green after a while and decomposition occurs. Under the same conditions, trimethylamine, piperidine, pyridine, picoline, quinoline and aniline give no color. Nor do extracts from putrefying horse-flesh and the entrails of animals, poisoned by arsenic or mercury, give the test, at least not when these extracts are prepared according to the Stas-Otto method.

5. **Physiological Test of H. Fühner.**¹—For the positive identification of nicotine, the best of all the numerous effects produced by this alkaloid is that upon the central nervous system, for it is exhibited by no other alkaloid related to it toxicologically, chemically or in action. As a result of this action, even a few minutes after injection of nicotine solutions a frog in a sitting posture will draw up its hind legs over the back so that the heels approach, touching when the action is stronger, or the legs even cross over the back. The results observed by Fühner, after injection of 0.2 mg. of nicotine hydrochloride into a frog weighing about 30 grams, were the following: soon after injection respiration ceased; instead of respiratory movements there was trembling and quivering of the flanks, the animal sat motionless, after about 5 minutes drawing up its hind legs over the back. The posture of the front legs is less characteristic than that of the hind legs. They usually lie downward on the belly. After 2-3 hours, respiration and spontaneous movements return. If 1 milligram of nicotine hydrochloride is injected, the typical posture of the hind legs comes to an end in about 30 minutes and they relax. As a result of these and larger doses of nicotine, the muscles of the animal acquire a characteristic condition of stiffness.

This effect upon the frog just described is entirely absent in the case of conium. A test with pure nicotine is advisable as a check.

ANILINE

Aniline, $C_6H_5.NH_2$, upon evaporation of the ether extract of the aqueous alkaline solution, appears in the form of yellow, reddish or brownish oil-drops. Dissolve some of this residue in water by shaking and apply to the solution the aniline tests already described on page 72. A further test for aniline consists in mixing some of the residue with a few drops of concentrated sulphuric acid and adding a few drops of potassium dichromate solution. If aniline is present, an evanescent blue color will appear.

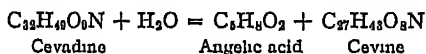
VERATRINE

Sabadilla seed, the seed of *Sabadilla officinalis*, contains several alkaloids, namely, cevadine or crystallized veratrine, $C_{28}H_{49}O_9N$,

¹ H. Fühner: *Detection and Estimation of Poisons Biologically*. E. Abderhalden's *Handbuch der Biochemischen Arbeitsmethoden* 1911, page 48.

veratridine or water-soluble, amorphous veratrine, $C_{33}H_{40}O_9N$, sabadine and sabadinine, $C_{27}H_{43}O_8N$. The first two bases are the principal constituents of officinal veratrine

Cevadine.—This base, having the formula $C_{32}H_{40}O_9N \cdot 3H_2O$, crystallizes in concentric clusters of needles readily soluble in boiling but only slightly soluble in cold alcohol. These crystals, at first clear and transparent, gradually become milky and opaque from loss of water. Its simple salts do not crystallize well but its gold double salt, $C_{32}H_{40}O_9N \cdot HCl \cdot AuCl_3$, forms yellow needles from hot alcohol and melts at 182° . Cevadine undergoes cleavage in hot alcoholic potassium hydroxide solution into angelic acid,¹ or into its stereo-isomer, tiglic acid, and the base cevine, $C_{27}H_{43}O_8N \cdot 3\frac{1}{2}H_2O$, which crystallizes well from dilute alcohol



Since cevadine forms with methyl iodide in ether solution cevadine-methyl iodide, having the composition, $C_{32}H_{40}O_9N \cdot CH_3I$, it is a tertiary base, and since it reacts with benzoic anhydride at 100° forming benzoyl-cevadine, $C_{32}H_{48}O_9N \cdot O \cdot CO \cdot C_6H_5$, and with acetic anhydride forming acetyl-cevadine, $C_{32}H_{48}O_9N \cdot O \cdot CO \cdot CH_3$, its molecule must contain one hydroxyl-group. Crystallized veratrine does not contain methoxyl-groups

Cevine forms a crystalline potassium compound having the composition, $C_{27}H_{43}O_8NK_2$, which serves for the detection of cevine as well as of crystallized veratrine. If alcoholic potassium hydroxide solution is added to an alkaline solution of cevine, separation of fine needles of this potassium compound begins at once. According to Hess and Mohr,² cevine is identical with sabadinine occurring in Sabadilla seed. In addition to the potassium compound, the bisulphate of cevine-sabadinine is especially characteristic. When calculated quantities of cevine-sabadinine and dilute sulphuric acid are brought together, a difficultly soluble crystalline precipitate separates at once. Upon recrystallization from hot water, this compound gives beautifully formed crystals having the composition $C_{27}H_{43}O_8N \cdot H_2SO_4 \cdot 2\frac{1}{2}H_2O$ and melting at 250° . From 210° on, coloration begins to appear. Upon treatment with benzoyl chloride in presence of sodium hydroxide solution, cevine and sabadinine give one and the same monobenzoyl-derivative

Cevine is less toxic than cevadine

According to M. Freund, cevine is converted by hydrogen peroxide into cevine oxide, $C_{27}H_{43}O_9N$, which crystallizes well and contains an additional atom of oxygen. This compound probably belongs to the class of amino-oxides, $R \equiv N=O$, for it is easily reconverted into cevine by sulphurous acid

¹ Angelic acid, or methyl-isocrotonic acid (I), and tiglic acid, or methyl-crotonic acid (II), are stereo-isomeric acids having the following formulae:



² K. Hess and H. Mohr: Cevine and Sabadinine. Ber. d. Deutsch. chem. Ges. 52 (1919), 1984

The method of E. Schmidt may be employed for the separation of crystalline veratrine from water-soluble veratrine in officinal veratrine. Dissolve the officinal preparation in a beaker in strong alcohol. Heat this solution to 60–70° and add enough warm water to produce a permanent turbidity. Cautiously add enough alcohol to clear the solution and allow evaporation to take place slowly at 60–70°. A white, crystalline precipitate will presently appear. Filter with suction, wash the precipitate with a little dilute alcohol, and recrystallize from hot alcohol. This is crystalline veratrine. Clear the filtrate from the crystalline precipitate by adding a little alcohol, and evaporate at 60–70°. This will give a second crop of crystals. By repeating this process several times, one may obtain in a crystalline condition about one-third of the veratrine taken. Finally evaporate the filtrate from the crystalline deposit at the given temperature until there is no longer any odor of alcohol. A considerable quantity of a resinous mass, which is a mixture of both alkaloids, will separate. The aqueous filtrate from this deposit will contain veratridine which may be obtained by rapidly evaporating the solution *in vacuo* over sulphuric acid.

Properties of Officinal Veratrine.—The pure officinal preparation is a white, amorphous powder, appearing crystalline under the microscope. It has a sharp, burning taste, and the minutest quantity introduced into the nostrils excites prolonged sneezing. It is almost insoluble in boiling water and the aqueous extract always has a faintly alkaline reaction. It is quite easily soluble in alcohol (1:4), ether (1:10), chloroform (1:2), as well as in benzene and amyl alcohol. All these solutions have a strong alkaline reaction. Officinal veratrine melts at 150–155° to a yellow liquid solidifying to a transparent, resinous mass. From its ether solution the alkaloid is usually deposited as a white, amorphous powder. In its reactions officinal veratrine behaves like cevadine and water-soluble, amorphous veratrine, for like these it neutralizes acids completely and the salts formed are as little crystalline as those of pure cevadine. Their solutions upon evaporation give water-soluble, resinous deposits having a bitter and at the same time sharp taste.

Toxic Action.—Veratrine gives rise to external burning and prickling of the skin and mucous membranes, and as a result of this action sneezing, watering of the eyes and roughness of the throat appear. In addition to these effects, internal administration of veratrine occasions the following symptoms: burning extending from the mouth to the stomach, flow of saliva and emesis, colic, bloody, mucous and watery stools, dizziness, delirium, irregular and retarded pulse. The central nervous system is first stimulated (raving, convulsions), then paralyzed. Death ensues following slowing of the pulse from paralysis of the heart and respiratory center. In experiments upon animals Kobert has established elimination of veratrine in the urine. In suspected veratrine poisoning, material suitable for examination is vomitus, gastric and intestinal contents, blood, organs rich in blood, and urine.

Detection of Veratrine

From a faintly acid solution ether extracts very little veratrine; but chloroform and amyl alcohol a considerable quantity. Ether and chloroform extract this alkaloid very readily from an alkaline

solution. The solution of veratrine, prepared with water containing sulphuric acid at a dilution of 1:5000, gives precipitates with phosphomolybdic acid, iodo-potassium iodide, tannic acid, potassium mercuric iodide, but shows only a slight turbidity with phosphotungstic acid and potassium bismuthous iodide. Gold chloride and picric acid barely cause precipitation of veratrine at a dilution of 1:1000. The following special tests may be used for recognition of veratrine:

1. Concentrated Sulphuric Acid Test.—Pour a few drops of concentrated sulphuric acid upon a trace of veratrine. The alkaloid will have a yellow color and, if stirred, give a yellow solution exhibiting a green-yellow fluorescence. Gradually this color will change to orange, then to blood-red and finally to cherry-red. Gentle heating will hasten this color change and veratrine, dissolved in concentrated sulphuric acid, will give a fine cherry-red solution almost immediately.

Froehde's and Erdmann's reagent give color changes similar to those caused by sulphuric acid.

2. Concentrated Hydrochloric Acid Test.—Warm a colorless solution of veratrine, prepared with 1-2 cc. of concentrated hydrochloric acid, for about 10 minutes in a small test-tube in a boiling water-bath. A cherry-red color will appear. This color will persist for several days and be quite distinct even with 0.2 mg. of veratrine.

3. Concentrated Nitric Acid Test.—This acid dissolves veratrine with a yellow color but the test is not characteristic.

4. Weppen's Test.—Thoroughly triturate 1 part of veratrine with about 5 parts of finely powdered cane-sugar. Stir and add a few drops of concentrated sulphuric acid. The mixture will first become yellow and after some time, beginning at the margin, change to grass-green and later to blue. Breathing upon the mixture will cause the color to change more quickly. Too great an excess of cane-sugar should be avoided.

In this test Laves¹ substitutes an aqueous furfural solution for cane-sugar. Mix 3-4 drops of 1 per cent. aqueous furfural solution with 1 cc. of concentrated sulphuric acid. Add 3-5 drops of this solution to the substance to be tested so that it just touches the edge of the liquid. If veratrine is present, a dark streak will gradually run from the substance into the liquid. At the starting-point it will appear blue or blue-violet and farther away green. If substance and liquid are stirred with a glass rod, the liquid will become dark

¹ E. Laves: Pharm. Ztg. 37, 328.

green After some time, or more quickly when warmed, the color will become blue and finally violet.

5. **Grandeau's Test.**—Direct addition of 1-2 drops of bromine water to the yellow solution of veratrine in concentrated sulphuric acid produces an immediate purple color almost identical with that appearing when the solution of alkaloid in concentrated sulphuric acid stands for a long time or is gently warmed.

6. **Vitali's Test.**—Dissolve veratrine in a few drops of fuming nitric acid and evaporate the solution to dryness upon the water-bath in a porcelain dish. A yellowish residue will remain. If this is cooled and then moistened with an alcoholic potassium hydroxide solution, the color will change to orange-red or red-violet and stirring will produce a solution having the same color. Atropine, hyoscyamine, scopolamine and strychnine respond to this test in a very similar manner.

7. **Physiological Test.**—Inasmuch as veratrine-like ptomaines have repeatedly been observed, it is often worth while to make a physiological test upon a frog in conjunction with the chemical examination. An aqueous solution of the ether residue should be prepared with the aid of very little acetic acid and injected subcutaneously into rather a large frog. If veratrine is present, movements occurring in the act of vomiting immediately appear. The number of the irregular pulse-beats drops to about half and less, from 60 to 30. With sufficiently large doses, tetanic spasms and symptoms of paralysis predominate from the start. If 0.01 mg of veratrine as the hydrochloride is injected into the thoracic lymph-sac of a frog, 20 minutes after the injection, according to H. Fühner, distinct symptoms of veratrine-action are in evidence. This is shown by the very stiff and clumsy manner in which the animal takes its first jump, which is brought about only by irritation, whereas the succeeding jumps are soon again normal. This "veratrine-effect" gradually develops and becomes even better. After an hour if the frog sitting at rest is irritated by pinching its toes, it laboriously makes the first sluggish jump after distending and stretching itself. The typical veratrine-effect is best produced by 0.03-0.06 mg of veratrine hydrochloride. After 0.1 mg symptoms of central irritation and paralysis appear in conjunction with muscular action. The striking stretching of the hind legs in jumping is even tetanus-like in character. Doses of 0.5-1 mg are fatal, following which the characteristic veratrine-effect is no longer distinctly in evidence and from the start symptoms of paralysis predominate (H. Fühner).

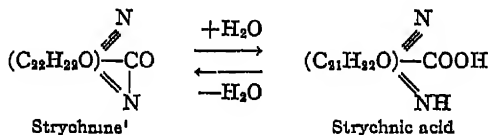
STRYCHNINE

Strychnine, $C_{21}H_{22}O_2N_2$, occurs together with brucine, combined with malic and caffetannic acid, in many *Strychnos* species. The seeds of *Strychnos nux vomica* (*Nux vomica*) contain 0.9-1.9 per cent. of strychnine and 0.7-1.5 per cent. of brucine. Total alkaloids

(strychnine + brucine) amount upon the average to 2.5 per cent. Ignatius beans contain upon the average about 1.5 per cent. of strychnine and about 0.5 per cent. of brucine.

Properties.—The free base strychnine forms colorless, shining prisms belonging to the rhombic system and melting at 268° . The alkaloid dissolves in 6600 parts of cold and 2500 parts of hot water, giving solutions having an alkaline reaction and a very bitter taste. It is nearly insoluble in absolute alcohol and absolute ether, soluble in 160 parts of cold and 12 parts of boiling alcohol (90 per cent. by volume), also soluble in commercial ether and benzene, but most readily soluble in chloroform (6 parts at 15°). Strychnine diluted with water 1:670,000 can be distinctly recognized by its very bitter taste.

Constitution.—Although strychnine contains 2 atoms of nitrogen in its molecule, it behaves like a monacid base, combining with one equivalent of acid and forming salts that are usually crystalline. These salts have a very bitter taste. The tertiary nature of this base is shown by the fact that strychnine combines with methyl iodide at 100° , forming strychnine iodo-methylate, $C_{21}H_{22}O_2N_2 \cdot CH_3I$, which with moist silver oxide forms an ammonium-base, the easily soluble strychnine methyl-ammonium hydroxide, $C_{21}H_{22}O_2N_2 \cdot CH_3OH$. If strychnine is heated with sodium methylate in alcoholic solution and then acidified with acetic acid, it yields microscopic crystals of strychnic acid. This behavior indicates that strychnine is probably the inner anhydride of strychnic acid and contains a group of the character of an acid amide (Tafel¹).



As a result of the action of hydrogen peroxide upon strychnine, strychnine oxide, crystallizing in colorless prisms, is formed together with other products of oxidation. This compound according to its properties belongs to the class of amino-oxides and, upon the basis of Tafel's strychnine formula, has the

formula, $\begin{array}{c} \text{OC} \\ | \\ \text{N} \end{array} (\text{C}_{20}\text{H}_{21}\text{O}) \equiv \text{N} \cdot \text{O}^2$. When strychnine is heated with water at 160 – 180° , it is converted into the isomeric iso-strychnine³ which has an action similar to that of curare. Strychnine, brucine, iso-strychnine and curare form a continuous series in which from the first to the last member the power of producing convulsions diminishes, whereas the action causing paralysis of the motor nerve-endings ("curare-action") increases.

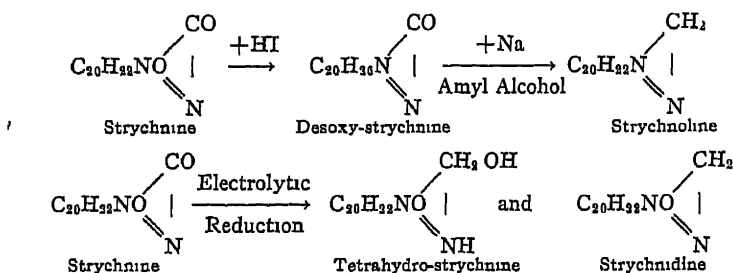
Reduction of strychnine by means of hydriodic acid converts it into desoxy-strychnine, $C_{21}H_{23}ON_2$, from which by treatment with sodium in boiling amyl

¹ J. Tafel: Strychnine. *Annalen d. Chemie* 264 (1891), 35.

² A. Pictet and Mattiesson: Strychnine Oxide. *Ber. d. Deutsch. chem. Ges.* 38 (1905), 2782.

³ Bacavescu and A. Pictet: Iso-strychnine. *Ber. d. Deutsch. chem. Ges.* 38 (1905), 2787.

alcohol solution is obtained the acid-free compound strychnoline, $C_{21}H_{26}N_2$. Electrolytic reduction of strychnine in strong sulphuric acid solution gives tetrahydro-strychnine, $C_{21}H_{28}N_2O_2$, and strychnidine, $C_{21}H_{24}ON_2$. These two bases may be separated by warm water, in which tetrahydro-strychnine is soluble and strychnidine nearly insoluble. Tetrahydro-strychnine and strychnidine are powerful convulsive poisons. According to Tafel, these reduction products of strychnine have the following formulae



Physiological Action.—Strychnine increases reflex irritability of the brain and spinal cord. Even the slightest stimulus, especially acoustic, optical, or tactile, may cause powerful reflexes after large doses of this alkaloid. Convulsions may follow each stimulus, if the dose is large enough. Very large doses of strychnine cause curare-like paralysis of the ends of motor nerves in frogs and warm-blooded animals. It may also affect the muscles of the heart. Strychnine diminishes the motile power of leucocytes and then arrests their motion. The poison also affects plant protoplasm, at least that of *Mimosa pudica*, in that the plant's motor organs lose their elasticity and flexibility.

The symptoms of strychnine intoxication, appearing after varying lengths of time but usually after 15–30 minutes, consist of stiffness of the neck, stiffness, lock-jaw, frothing at the mouth, cessation of respiration, and especially tetanus which appears in repeated attacks every 10–15 minutes. The eyeballs protrude from their sockets during these attacks and the pupils are enlarged. There is severe cyanosis but this disappears as the attack subsides. Emesis rarely occurs. Sometimes the attacks appear to be spontaneous, at other times they are the result of external stimuli, noises, slight movements, flashes of light from a reflector. Individual attacks last 3–4 minutes and death usually occurs at the third or fourth attack, within a period of 10 minutes to 3 hours, as the result of asphyxiation. The rapidity with which *rigor mortis* appears is quite noticeable.

Behavior of Strychnine in the Animal Body and Elimination

Absorption of strychnine from the intestinal tract probably proceeds rather slowly, since in cases of poisoning most of this alkaloid, is found in the stomach and sometimes also in the intestines. According to Bakunin and Majone,¹ the amount of strychnine found in the organs of animals is usually very small and rarely exceeds a tenth of the quantity administered. In dogs elimination of

¹ W. Bakunin and V. Majone. Toxicological Experiments with Strychnine. *Gaz chim ital* 36 (1905), II, 227.

strychnine in the urine is at an end 72 hours after administration. According to Salant,¹ strychnine undergoes in the intestines of animals changes of such a character that it can no longer be recognized as such by chemical tests, whereas even 1 mg. of the alkaloid in the stomach of the same animals can be distinctly recognized. From the results of these experiments it follows that stomach and intestines should be examined separately, if it is a matter in legal cases of testing for strychnine in the cadaver. A. de Dominicus² maintains as the result of experiments upon animals that strychnine may also be detected in the bones of animals poisoned by this alkaloid, even when the quantities of the poison are small. The bones examined were carefully freed from all soft parts, then finely ground, and tested for strychnine by the Stas-Otto method. Elimination of strychnine from the organism, aside from saliva, bile and milk, takes place mainly in the urine, and in the case of man unaltered in form. Elimination begins even in the first hour, is slight after 2 days, and ceases entirely much later. In small doses the percentage of strychnine eliminated unchanged in the urine is less than after larger doses, in which case 70-75 per cent of it remains undestroyed. Strychnine can be stored up unchanged in the liver, kidneys, brain and spinal cord (see above).

According to experiments made by Hatcher and Eggleston³ upon dogs, cats and guinea-pigs, toxic but not lethal doses of strychnine, amounting in all to 25 times that of a single lethal dose, may be administered at short intervals in the course of 12 days without lasting ill-effects. Only a small part of the strychnine administered could be found again in the urine, elimination through this channel ceased within 24-48 hours. Strychnine could not be detected in the faeces. A very large part of the strychnine taken was rapidly destroyed in the animal organism. Only when death ensued within 3 hours after the last dose of strychnine, was it possible to find this alkaloid in the tissues.

The stability of strychnine in the cadaver seems to be as certain as the rapidity with which most of it is destroyed in the living organism as a result of animal metabolism, for Cram and Meserve⁴ found unchanged strychnine in the liver and spinal cord of the cadaver of a man exhumed after 4 months. Kratter was able to detect it positively even after 6 years. Consequently strychnine sometimes resists putrefaction in cadavers for quite a long time.

Cadaveric Strychnine.—Mecke⁵ isolated from a cadaver, that had already begun to putrefy, a crystalline ptomaine very much like strychnine in its chemical behavior. This ptomaine, however, differed from strychnine in having only a faintly bitter taste, in being non-toxic, and in exerting no tetanizing action upon frogs.

¹ W. Salant. *Zentralbl. f. inn. Med.* 23, 902.

² A. de Dominicus. *Detection of Strychnine in Bones*. *Vierteljahrsschr. f. gerichtl. Med. u. Sanitätsw.* 28 (1904), 284.

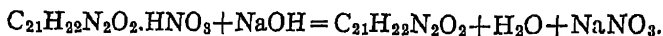
³ R. A. Hatcher and C. Eggleston: *Fate of Strychnine in the Body*. *Journ. Pharmacolog. and Exper. Therapeutics* 10 (1917), 281.

⁴ M. P. Cram and P. W. Meserve: *Stability of Strychnine in the Cadaver*. *Journ. of Biol. Chem.* 8 (1910), 495.

⁵ Mecke and Wimmer: *A Cadaveric Alkaloid resembling Strychnine*. *Pharm. Ztg.* 43 (1898), 300.

Detection of Strychnine

Potassium and sodium hydroxide, ammonia and alkali carbonates precipitate the free strychnine base from aqueous solutions of its salts as a white, crystalline solid.



Ether will extract strychnine from an alkaline solution and deposit the alkaloid upon evaporation in fine crystalline needles. Chloroform takes up the alkaloid more freely, since strychnine is considerably more soluble in this solvent than in ether. Even very dilute solutions of strychnine salts give precipitates with most of the alkaloidal reagents. Tannic acid, potassium mercuric iodide and phospho-tungstic acid produce white precipitates, gold chloride and phospho-molybdic acid yellow precipitates, and iodo-potassium iodide a brown precipitate. To obtain tests with these reagents, the residue from ether should first be dissolved in very dilute hydrochloric acid.

Concentrated sulphuric acid, Erdmann's and Froehde's reagent dissolve perfectly pure, brucine-free strychnine without color.

Concentrated nitric acid dissolves strychnine with a yellowish color. Potassium dichromate, added to aqueous solutions of strychnine salts, precipitates strychnine dichromate, $(\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2)_2 \cdot \text{H}_2\text{Cr}_2\text{O}_7$, in the form of fine, yellow, crystalline needles which upon recrystallization from hot water appear as shining orange-yellow needles.

Potassium ferricyanide, added to solutions of strychnine salts, precipitates golden yellow, crystalline strychnine ferricyanide, $(\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2)_2 \cdot \text{H}_3\text{Fe}(\text{CN})_6 \cdot 6\text{H}_2\text{O}$.

Special Reactions

1. **Sulphuric Acid-Dichromate Test.**—Dissolve a trace of strychnine in 2–3 drops of concentrated sulphuric acid upon a watch-glass. Add a particle of potassium dichromate and hold it firmly in one place upon the glass. Intense blue or blue-violet streaks will come from the potassium dichromate, if the watch-glass is tilted up and down. If the entire mixture is stirred, the sulphuric acid will have a beautiful evanescent blue or blue-violet color.

This test may also be made by scattering upon the surface of the solution of strychnine in concentrated sulphuric acid a few particles

of coarsely powdered potassium dichromate and mixing well with a glass rod. In this way the blue to blue-violet color-reaction is given very well. The blue color is not permanent. It soon changes to red and finally to dirty green.¹

Strychnine dichromate and ferricyanide give this test especially well. To convert the residue from the ether extract of the aqueous alkaline solution into the former salt, pour a very dilute potassium dichromate solution over the residue. When the two substances have been in contact for some time, pour off the remaining liquid. Wash the product once with a little water thoroughly draining off the water. Put some of the residue upon the end of a glass rod and draw it through a few drops of concentrated sulphuric acid upon a watch-glass. If strychnine is present, blue and violet streaks will appear. The test may also be made by moistening the residue direct with a few drops of concentrated sulphuric acid.

Other oxidizing agents may be substituted for potassium dichromate, such as potassium permanganate, lead peroxide, manganese dioxide, potassium ferricyanide, cerium oxide and vanadic acid. But neither potassium nitrate nor nitric acid can be used, as these reagents even prevent this test. Consequently strychnine nitrate does not give the test.

Mandelin's reagent,² that is, vanadic-sulphuric acid, gives this strychnine test very well. The blue or violet color given by this reagent with strychnine is more permanent than that produced by potassium dichromate. The color finally changes to orange-red.

Sonnenschein's reagent³ dissolves strychnine with a blue color gradually changing to red.

2. Wharton's³ Test.—Dissolve the substance to be tested in a dry condition in chloroform. Put this solution in a small test-tube and evaporate the chloroform by setting the tube in a larger one containing boiling hot water. When the substance is dry or nearly so, add a few drops of a mixture of equal parts of strong sulphuric acid and water and dissolve by shaking. Now introduce bromine vapor carefully and move the tube to and fro so that the solution takes up bromine. Replace the tube in boiling water to expel excess of bromine vapor. If strychnine is present, a carmine-red color

¹ According to Tafel (*Ann. d. Chemie* 268 (1892), 233), this color-reaction is characteristic of many anilides and is due to the presence of the group —CO—N= .

² See "The Preparation of Reagents" page 640.

³ J. C. Wharton. Test for Strychnine with Bromine. *Journ. Pharm.* 8 (1901), 201.

will appear in a few minutes, increasing in intensity as the bromine evaporates. This color fades after a time. Instead of bromine vapor, a solution of a drop of bromine in 2 cc. of chloroform may be used. If the quantity of strychnine present is small, only a little bromine should be added to the solution.

3. **Physiological Test.**—Dissolve the ether residue in a few cc. of very dilute hydrochloric acid. Evaporate the filtered solution to dryness upon the water-bath. Dissolve the residue in pure water (about 1 cc.) and inject the solution into the lymph-sac of a lively frog. Then put the frog in a large, loosely covered beaker. Toxic symptoms will appear in 5–30 minutes, depending upon the quantity of strychnine. This alkaloid does not increase reflex irritability for all kinds of stimuli but only for tactile, optical and especially for acoustic stimuli. When the dose of strychnine is sufficiently large, each kind of stimulus mentioned will produce convulsions like those caused by tetanus. For example, if the beaker containing the “strychnine-frog” is gently tapped, this slight acoustic stimulus is sufficient to produce convulsions. The convulsions produced in a frog by strychnine, contrary to those caused by picrotoxin, are characterized by a prevailing extended position of the hind legs. According to H. Fühner, between 0.02–0.05 mg. of strychnine nitrate is the smallest quantity capable of producing tetanic convulsions in a medium-sized frog. With 0.05 milligram the animals show increased reflex irritability, the first tetanic attacks following after 20–30 minutes upon external stimulus, especially acoustic. At first they are of short duration, as the animals again draw up their hind legs after the convulsion. Later the legs are kept continuously extended and convulsions occur without external stimulus. During these convulsions the front legs are crossed. With doses of about 1 mg. of strychnine nitrate paralysis, in conjunction with irritability and following close upon it, begins to predominate but this is striking only in water-frogs.

Detection of Strychnine in Presence of Brucine

More than traces of brucine may entirely prevent or interfere with the detection of strychnine with concentrated sulphuric acid and potassium dichromate. Sometimes Mandelin's reagent will show strychnine in presence of brucine. For the more certain detection of strychnine, dissolve the ether residue containing brucine in about 2 cc. of dilute sulphuric acid, add 2 drops of concentrated nitric acid, and allow the mixture to stand 3–4 hours in the cold. Render strongly alkaline with sodium hydroxide solution and extract thoroughly with ether. The residue from ether will be brucine-free or nearly so. In case the material contains strychnine, it will give very satisfactory tests with concentrated sulphuric acid and potassium dichromate, as well as with Mandelin's and Sonnenschein's reagent. Under the experimental conditions described, the easily oxidized brucine, but not the more resistant

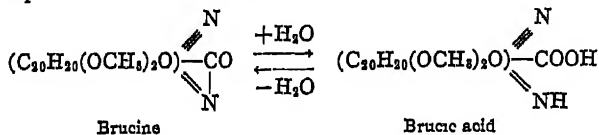
strychnine, is destroyed by oxidation. Extraction of the alkaline solution with ether removes strychnine but not the oxidation products of brucine

BRUCINE

Brucine, $C_{23}H_{28}N_2O_4 \cdot 4H_2O$, always occurs together with strychnine in the seeds of *Strychnos nux vomica* and in Ignatius beans. The bark of *Strychnos* species, however, appears to contain together with brucine only traces of strychnine.

Properties.—Brucine crystallizes upon evaporation of its dilute alcoholic solution in colorless, transparent, monoclinic plates with 4 molecules of water of crystallization. Brucine loses part of its crystal water in dry air and all *in vacuo* over sulphuric acid, as well as when heated at 100° . From strong alcohol it crystallizes with 2 molecules of crystal water. This hydrated brucine melts somewhat above 100° in its crystal water. The anhydrous alkaloid melts at 178° . Brucine belongs to those alkaloids that are relatively easily soluble in water. Crystallized brucine dissolves in 320 parts of cold and 150 parts of boiling water, giving an alkaline, laevo-rotatory solution having a very bitter taste. This alkaloid is easily soluble in alcohol (1:2), and also in acetone and chloroform; in absolute ether on the other hand it is almost insoluble.

Constitution.—Brucine is a rather strong, monacid base, forming salts with one equivalent of acid that usually are crystalline. Caustic alkalis and alkaline carbonates precipitate brucine from aqueous solutions of these salts. Ammonia in excess first exerts a solvent action upon brucine but gradually it separates from solution in crystalline form. Brucine nitrate, $C_{23}H_{28}N_2O_4 \cdot HNO_3 \cdot 2H_2O$, crystallizes in prisms. Since brucine combines with methyl iodide, forming brucine iodo-methylate, $C_{23}H_{28}N_2O_4 \cdot CH_3I$, the tertiary nature of this alkaloid is shown. By means of Zeisel's method 2 molecules of methyl iodide can be obtained with hydriodic acid. Consequently brucine contains two methoxyl-groups. Brucine, heated at 80° with a solution of sodium in absolute alcohol, and also with alcoholic sodium hydroxide, yields upon acidification with acetic acid brucic acid, $C_{23}H_{26}N_2O_5 \cdot H_2O$, the molecule of which has been formed by the addition of a molecule of water. Since this acid forms a nitrosamine with nitrous acid, one imino-group (NH) must be present. Tafel and Moufang¹ express the relationship between brucine and brucic acid as follows.

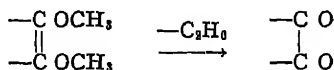


Heated with water, brucic acid is converted into brucine. Consequently brucic acid is related to brucine as strychnic acid is to strychnine.

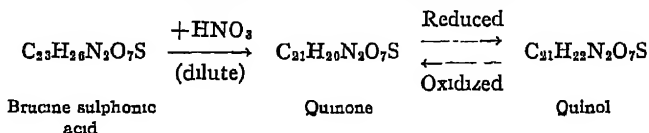
Hydrogen peroxide converts brucine into the beautifully crystalline brucine oxide, $C_{20}H_{20}(OCH_3)_2N(CO \cdot N) \cdot N \cdot O$, which is reduced by sulphurous acid to brucine. Brucine oxide therefore belongs to the class of amino-oxides

¹ N. Moufang and J. Tafel. Brucine Ann. d. Chem. 304 (1898), 24.

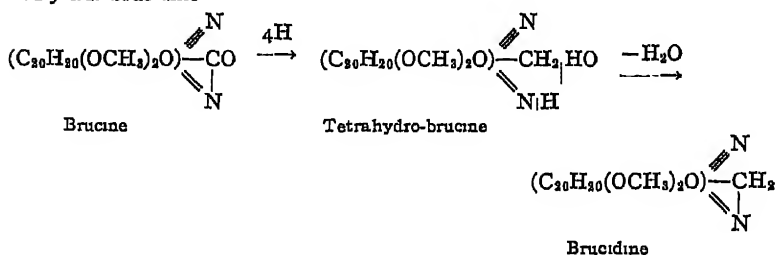
Action of Nitric Acid.—When brucine is boiled with 5 per cent nitric acid and the product of the reaction is decomposed with sodium acetate, nitro-brucine hydrate, $C_{21}H_{21}(NO_2)(NCH_3)_2N_2O_8$, crystallizing in gold-yellow leaflets is formed. By the action of concentrated nitric acid (sp gr 1.4 = 61 per cent.) brucine is decomposed with cleavage of 2 molecules of methyl nitrite, CH_3ONO , which breaks into NO and CO_2 . Brucine gives the so-called cacoteline, $C_{21}H_{21}(OH)_2(NO_2)N_2O_8 \cdot HNO_3$, which is precipitated by water orange-yellow and can be recrystallized from water containing nitric acid. It is also formed by warming brucine with 20 times the quantity of 20 per cent nitric acid at 60–70°. Cacoteline is the nitrate of bi-desmethyl-nitro-brucine hydrate, $C_{21}H_{21}(OH)_2(NO_2)N_2O_8$, obtained by dissolving cacoteline in sodium hydroxide solution and neutralizing with acetic acid. The probable explanation of the conversion of brucine into cacoteline is that the two methoxyl-groups of brucine give rise to quinone-formation ¹



Isolation of the quinone is more easily accomplished by starting with brucine sulphonic acid than with brucine itself. Like all quinones it is reduced by sulphurous acid to the corresponding colorless quinol which is converted back into quinone by cold dilute nitric acid (Leuchs and Geiger)



Reduction.—Electrolytic reduction in sulphuric acid solution converts brucine into tetrahydro-brucine which loses water when heated in a current of hydrogen and yields brucidine



Physiological Action.—The power of brucine to give rise to tetanic convulsions is about 40–50 times less than that of strychnine. Its curare-like action, however, is stronger than that of strychnine. Following internal administration, elimination of brucine runs parallel with absorption so that cumulative action is not to be feared as in case of strychnine.

¹ H. Leuchs and R. Anderson: Contribution to the Knowledge of Brucine. The Nitric Acid Reaction (Strychnos Alkaloids XI) Ber d Deutsch chem. Ges 44 (1911), 2136

Detection of Brucine

Ether, benzene or chloroform will extract brucine from an aqueous alkaline solution. Owing to its slight solubility, several extractions with ether are necessary. Evaporation of the ether extract usually leaves the alkaloid in an amorphous condition. The sensitiveness of the alkaloidal reagents toward brucine is as follows: iodo-potassium iodide (1.500,000), potassium mercuric iodide (1.30,000), gold chloride (1.20,000), potassium bismuthous iodide (1.5000), tannic acid and phospho-molybdic acid (1:2000); and platinum chloride (1 1000). Brucine picrolonate forms cubic or rhombic crystals that darken at 254° and melt at 256°. The following tests may be employed for the more certain recognition of brucine:

1. **Nitric Acid-Stannous Chloride Test.**—Concentrated nitric acid dissolves brucine and its salts with a blood-red color, at once changing to red-yellow and finally to pure yellow. Add a few drops of freshly prepared, dilute stannous chloride solution to this yellow-red or yellow solution in a test-tube. A fine violet color will appear. If the solution is now warmed and a drop of nitric acid added, usually the red-yellow color reappears and further addition of stannous chloride solution restores the splendid violet color. The smaller the quantity of nitric acid, the more likelihood that this test will give a good result.

2. **R. Mauch's Modification of Nitric Acid-Stannous Chloride Test.**—An excellent result may be obtained with this test in the following manner. Dissolve brucine in 60 per cent aqueous chloral hydrate solution and put about 0.5 cc. of this solution into a test-tube. Add very little dilute nitric acid and thoroughly mix the two solutions. Add this mixture to 3 times its volume of concentrated sulphuric acid so that the former is on the surface. A yellow-red to deep red zone will appear immediately. When the upper layer becomes yellow, introduce from a pipette a little dilute stannous chloride solution as an upper layer. A fine violet zone will appear between the two upper layers. The intensity of this color will gradually increase, especially if the test-tube is gently tilted to and fro. Prepare the stannous chloride solution by dissolving 1 part of stannous chloride, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, in 9 parts of hydrochloric acid having a specific gravity of 1.12 (about 24 per cent HCl).

ATROPINE

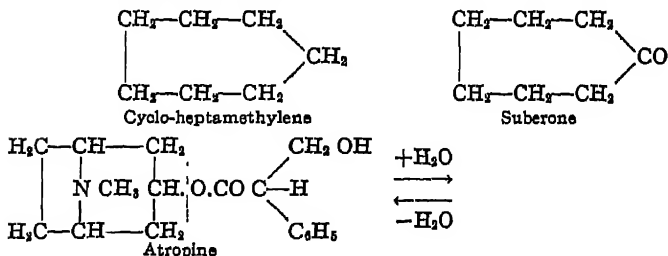
The belladonna plant, *Atropa Belladonna*, contains alkaloids in its various parts. These consist almost exclusively of hyoscyamine,

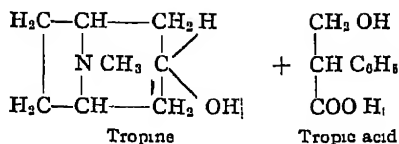
that is, in the leaves up to 0.4 per cent., in the unripe, dried fruit 0.8 per cent. (ripe 0.4 per cent.), and in the root 0.5 per cent. of alkaloid. The thorn-apple, *Datura Stramonium*, contains hyoscyamine and not atropine. Atropine obtained from the different parts of the plant is formed, at least most of it, as a result of molecular rearrangement, that is, by conversion of hyoscyamine into its racemic form.

Properties.—Atropine, $C_{17}H_{23}NO_3$, crystallizes in shining pointed needles, melting at 115° and dissolving in 600 parts of water, 50 parts of ether, and 3.5 parts of chloroform. It is also soluble in alcohol, amyl alcohol and benzene. The aqueous solution of the alkaloid is alkaline and has a persistent, unpleasant, bitter taste. Unlike the optically active hyoscyamine, atropine is inactive. Atropine is volatile in small quantity with steam.

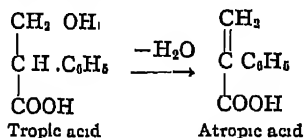
Constitution.—Atropine is a strong, monacid base, neutralizing acids with formation of salts that do not always crystallize well. A fact to be noted is that aqueous solutions of atropine salts in time undergo decomposition. Atropine sulphate, used in medicine, has the formula, $(C_{17}H_{23}NO_3)_2H_2SO_4 \cdot H_2O$, since atropine, heated in ether-alcohol solution in sealed tube at 100° with ethyl iodide, gives atropine iodo-ethylate, $C_{17}H_{23}NO_3 \cdot C_2H_5I$, from which silver oxide sets free atropine-ethyl hydroxide, $C_{17}H_{23}NO_3 \cdot C_2H_5OH$, the tertiary character of this alkaloid is shown. From the fact that atropine gives, together with other substances, methyl amine, CH_3NH_2 , when heated with soda lime, a methyl-group must be attached to nitrogen, that is, the molecule contains the NCH_3 group. Moreover atropine is an ester, for it is hydrolyzed by fuming hydrochloric acid, slowly at ordinary temperature but rapidly and completely at $100-130^\circ$, into tropine which is both a secondary alcohol and tertiary base, and tropic acid. A portion of the tropic acid first formed loses water forming atropic acid. Longer boiling with barium hydroxide solution will also bring about this reaction.

Atropine may be regarded as a derivative of cyclo-heptamethylene, for it can be made to give suberone. Starting from the latter, R. Willstätter (1901) synthesized tropine and thus was able to synthesize atropine itself. Since the constitution of tropic acid is also known as the result of synthesis, that of atropine is established beyond question. To reverse the process of hydrolysis, or saponification of the ester atropine, and re-form the ester, it is necessary to heat tropic acid and tropine for rather a long time upon the water-bath with 20 times the quantity of 5 per cent. hydrochloric acid. The following formulae serve to illustrate the reactions mentioned above.





Conversion of tropic into atropine acid.



Hyoscyamine is stereo-isomeric with atropine. Optically active hyoscyamine, $[\alpha]_D^{20} = -20.97^\circ$, is changed into inactive atropine, when heated at 110° with exclusion of air, or when its alcoholic solution is allowed to stand in the cold with a few drops of sodium hydroxide solution until it has become optically inactive.

Physiological Action.—Atropine produces paralysis in different peripheral organs. Moreover it acts upon the central nervous system, especially in larger doses, increasing the irritability of the brain, that is, it first causes irritability and then paralysis. Among the peripheral organs upon which atropine acts is the eye, in which it causes enlargement of the pupils and destroys the possibility of accommodation for objects close at hand. Enlargement of pupils after atropine has been dropped into the eye is most marked in human beings and cats and less in rabbits and dogs. In addition the secretion of almost all glands is suppressed by atropine or at least very much diminished. Stimulation of an animal treated with atropine elicits no secretion of saliva from the submaxillary gland. Under the influence of atropine, secretion of sweat and mucus ceases. The pancreatic secretion increased by muscarine is suppressed by atropine, that of the bile diminished. Upon this property of depressing glandular secretions is based the therapeutic application of atropine for night-sweats and symptoms of poisoning. Since atropine like hyoscyamine is easily absorbed, the first symptoms of intoxication usually appear in a few minutes. Among the symptoms that manifest themselves are: dryness of the mouth and throat, hoarseness, difficulty in swallowing, or it becomes wholly impossible, bright redness and swelling of the face, quickening of the pulse, skin dry, scarlet-red and hot. Further there is bulging of the eye-balls, enlargement and immovability of the pupils, in conjunction with many disturbances of vision, such as weakness of sight and loss of accommodation for objects near at hand. Still other symptoms mentioned by Kobert are: Headache, dizziness, hallucinations, delirium and raving, manifesting themselves in running, dancing, foolish talk, laughter and falling down. These severe symptoms of irritation do not appear until a later stage of poisoning. In case of poisoning by hyoscyamine free from atropine this stage of irritation is almost entirely lacking. The typical mydriatic action of atropine and hyoscyamine is not noticeably evident in the case of their two cleavage-products, tropine and tropic acid. Both mydriatics are easily absorbed and within a comparatively short time are eliminated for the most part unchanged in the urine. On several occasions in poisonings that did not end fatally, Kratter was able to get a distinct

test for atropine in urine Unquestionably a portion of the atropine absorbed is destroyed in the organism. Experiments made by Gonnermann,¹ showing that the liver, pancreatin and trypsin cause cleavage of atropine, are in favor of this view Pepsin had no action, nor bacterium coli communis and the contents of human intestines

Stability of Atropine during Cadaveric Putrefaction

Atropine, whether taken as the pure alkaloid or in the form of deadly nightshade, is rapidly absorbed from all parts of the body and disseminated in the body by the blood-stream in proportion to the distribution of the blood Elimination begins quickly and takes place through all the channels of elimination of the body In man and animals, as a result of its specific action in paralyzing secretions, elimination of atropine extends over a longer period, lasting 4-5 days in man after poisoning from 3-5 *Atropa berries*, and in a dog 14 days after subcutaneous injection of 0.5 gram of atropine sulphate (Ipsen²) After very thorough investigations, contrary to the observations and views of other analysts, Ipsen came to the conclusion that atropine can still be detected in parts of cadavers after a rather long time He succeeded in detecting this alkaloid even after 12 years when a quantity of 0.03 gram of sulphate in 300 cc each of blood, urine and beer, or of pure alkaloid in 300 cc of blood, was exposed to the effects of decomposition at 35° Atropine therefore is extraordinarily resistant to putrefaction In the viscera of a cadaver exhumed after 3 years, Ipsen detected atropine in quantities that could be estimated, and identified it as such with certainty by tests upon the human eye

Material suitable for examination is gastric and intestinal contents, organs rich in blood, blood and especially urine When poisoning has been caused by parts of the plant, search for them should be made in the gastric and intestinal contents Even after they have left the intestines, seeds still retain a portion of the alkaloid having mydriatic action According to Ipsen, one such seed is said to be sufficient, after it has been cleaned, ground and extracted with very slightly acidulated water, to give a solution that will produce the characteristic enlargement of the pupil when introduced into the eye

Detection of Atropine

Ether, benzene or chloroform will extract atropine from an aqueous solution rendered alkaline with sodium hydroxide In a special search for atropine, homatropine or scopolamine, when morphine need not be considered, render the aqueous tartaric acid solution alkaline with sodium carbonate, or better bicarbonate, and extract with chloroform or ether, since sodium hydroxide may saponify these mydriatic alkaloids which are esters Upon evaporation of

¹ M. Gonnermann. Capacity of Liver Histozyne and Some Enzymes to Cause Cleavage of Some Glucosides and Alkaloids. *Pflüger's Archiv* 113 (1906), 168.

² C. Ipsen. Detection of Atropine. *Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw.* 31 (1906), 308

the ether solution, atropine usually appears as a non-crystalline varnish. Even very dilute solutions of atropine and hyoscyamine give precipitates with most of the general alkaloidal reagents. R. Eder¹ has tested the sensitiveness of these reagents to atropine and hyoscyamine, dissolved in 0.5 N-sulphuric acid. Precipitation was made upon watch-glasses and 0.5 cc. of the alkaloid solution and 2 drops of the reagent were used for each test. The period of observation was 2-3 minutes. Atropine and hyoscyamine showed the same limits of precipitation where turbidity or faint precipitation could just be seen, that is: iodo-potassium iodide 1:200,000, phosphotungstic acid: 1:100,000, potassium bismuthous iodide 1:100,000, phosphomolybdic acid 1:70,000; potassium mercuric iodide 1:10,000, silico-tungstic acid 1:20,000, gold chloride 1:1000. Iodo-potassium iodide produces in aqueous solutions of atropine salts a red-brown precipitate that changes within 5-10 minutes into blue-green, shining, metallic leaflets of the hydriodide of atropine-tetradide, $C_{17}H_{23}NO_3 \cdot I_4 \cdot HI$. This is characteristic of atropine, especially upon microscopic examination.

Special Reactions

1. **Vitali's² Test.**—Evaporate upon the water-bath in a porcelain dish atropine or an atropine salt with a few drops of fuming nitric acid. Moisten the yellowish residue when cold with a few drops of a solution of potassium hydroxide in absolute alcohol (about 1:10). An evanescent violet color at once changing to a fine red will appear.

In addition to atropine, hyoscyamine, scopolamine and eumydrine³ also give Vitali's test. Pseudo-aconitine gives with nitric acid a yellow residue that becomes purple-red when moistened with alcoholic potassium hydroxide. Vitali has further shown that the succeeding red color is not characteristic, since strychnine gives a fine red or a transitory violet color. Veratrine and apomorphine also give colors with Vitali's test that might be mistaken for those given by atropine. According to Pohl, physostigmine and pilocarpine also interfere with Vitali's atropine test. By extracting the alkaline aqueous solution with pure carbon disulphide, atropine which is alone soluble can be separated from the other two insoluble alkaloids.

¹ R. Eder: Reactions for the Detection of Atropine and Other Related Mydriatic Alkaloids. Schweizer Apotheker-Zeitung 1916

² Vitali: L'Orosi 1880 No. 8 and Archiv d. Pharmaz. (3) 18 (1881) No. 37, 307

³ Eumydrine, formed by treating atropine methyl bromide with silver nitrate, is atropine methyl nitrate, white crystals soluble in water and alcohol and melting at 163°:

$$C_{17}H_{23}NO_3 \cdot CH_3Br + AgNO_3 = AgBr + C_{17}H_{23}NO_3 \cdot CH_3NO_3 \text{ (Eumydrine).}$$

2. **Gulielmo's¹ Odor Test.**—Heat a little atropine in a small, dry test-tube over a low flame until a white vapor appears. At the same time an agreeable odor, recalling the perfume of black-thorn blossoms, can be detected. Then add about 1 cc. of concentrated sulphuric acid and heat until the acid begins to darken. Without cooling, dilute at once with 2 cc of water added from a small graduate. During foaming this odor will be still stronger. A positive test is given by 0.01 gram of atropine. To test the residue from the ether extract of the aqueous alkaline solution, dissolve it in a little ether, evaporate this solution in a test-tube put in a warm water-bath, and examine the residue. Mandelic acid derivatives, homatropine (see page 182) and euphthalmine, give the odor of benzaldehyde in this test, whereas the flower perfume of the tropic acid derivatives is said to be due to the cleavage-product atropic acid (Gadamer), or styrole (Kondakow), or phenyl-acetaldehyde (Rosenthaler).

3. **Color Reactions.**—Atropine differs from most of the alkaloids in not giving, at least in the cold, any striking colors that are characteristic with reagents usually employed for this purpose, such as concentrated sulphuric and nitric acid, and the reagents of Froehde, Erdmann, Marquis and Mecke. According to Wasicky,² perhydrosulphuric acid and p-dimethyl-amino-benzaldehyde-sulphuric acid (dimethyl-amino-benzaldehyde = $(\text{CH}_3)_2\text{N} \cdot \text{C}_6\text{H}_4 \cdot \text{CHO}$) are exceptions.

(a) **Perhydrol-Sulphuric Acid (Schaer's¹ Reagent) Test.**—Atropine, hyoscyamine, homatropine, scopolamine and cocaine give a fine color-reaction with this reagent. If a few drops of perhydrosulphuric acid are added to a particle of the alkaloid, after 0.5 minute an intense leaf-green color beginning at the margin appears, becoming olive-green after a few minutes and finally discolored or brown-green.

(b) **Para-dimethyl-amino-benzaldehyde-Sulphuric Acid Test.**³—Add a drop of the reagent to a small quantity of atropine, hyoscyamine or scopolamine and warm gently, best upon an asbestos-plate. A red color appears, becoming in time an intense cherry-red to violet and remaining unchanged for a day. According to Wasicky, this is as delicate as the physiological test. Homatropine, cocaine and tropacocaine do not give this reaction

¹ *Zeitschr f analyt Chemie* 1863, 404

² R. Wasicky. A New and very Delicate Color Reaction for Atropine, Hyoscyamine and Scopolamine. *Zeitschr f analyt Chemie* 54 (1915), 393.

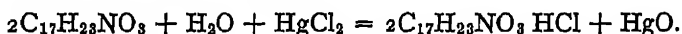
³ See under "Preparation of Reagents" page 641.

Morphine and codeine even in the cold give at once a bright red color but it lacks the violet tone. Atropine gives this color in the cold only after a few hours. Quinine gives a red-brown color, physostigmine leaf-green, narcotine and papyaverine orange, and veratrine deep green upon gentle warming

4. Alkaline Reaction Test.—In aqueous solution, atropine, hyoscyamine and homatropine turn red litmus paper blue and also react with phenol-phthalein. Place a trace of alkaloid upon a strip of phenol-phthalein paper, add a drop of absolute alcohol, and allow it to evaporate. No color will appear. Then add a drop of water. The paper will at once turn red.

Cocaine, morphine, codeine and pilocarpine turn red litmus paper blue but have no action upon phenol-phthalein paper. Obviously, alkalies or alkaline carbonates should not be present. In case the ether residue contains the latter, it should be redissolved in the purest chloroform, the solution passed through a dry paper and then evaporated. This residue may then be tested with phenol-phthalein paper.

5. Gerrard's¹ Test.—Add about 2 cc. of a 1 per cent solution of mercuric chloride in 50 per cent. alcohol to 1 milligram of atropine. Warm very gently and a yellow precipitate of mercuric oxide, becoming red upon boiling, will appear:



Hyoscyamine and homatropine also give Gerrard's test. This reaction fails with too great dilution.

In this test brucine, strychnine, veratrine, aconitine, coniine, caffeine and quinine give white precipitates. Those produced by morphine, and codeine are pale yellow.

6. Eder's² Micro-Tests with Bromine Water and Bromo-Potassium Bromide.³—Place a drop of a solution of the alkaloid in 0.5 n-sulphuric acid upon a glass slide and then allow a drop of the reagent to fall into this solution. The mixture should not be stirred. Several minutes after precipitation, put on a cover-glass without disturbing crystal formation. A microscope magnifying 180-250 times should be used in studying the forms of crystals. In the test given by atropine with saturated bromine water, the rather large prisms are especially characteristic. These are usually pale yellow, flat rods very regularly formed with the ends frequently cut off

¹ A. W. Gerrard: *Pharm. Journ. and Trans.* (3) 14 (1884), 718.

² R. Eder: *Loc. cit.* page 471.

³ See "Preparation of Reagents" page 641.

obliquely The test with bromo-potassium bromide is carried out in the same manner as that with bromine water and is more delicate than the latter. With a magnification of 200 times, small, pale yellow needles, spindle to cylindrical in form, also frequently in star or Y-shaped clusters, may be seen. Bromine water and bromo-potassium bromide also produce characteristically shaped crystalline precipitates in hyoscyamine and homatropine solutions. For purposes of comparison, tests should always be made with pure alkaloids at the same time Scopolamine gives yellow precipitates with both bromine reagents Under the microscope they are seen to consist of yellow amorphous droplets Only as exceptions do centers of crystallization appear in spots

Since atropine and hyoscyamine sublime without decomposition *in vacuo*, according to Eder micro-sublimates¹ may also be employed in testing for these two bases which are especially suited for this micro-chemical test. In experiments with the bromine reagents, bromine gradually evaporates and with it crystals disappear completely Addition of another drop of bromine water or bromo-potassium bromide to the clear colorless solution will cause anew the characteristic precipitations

In addition to the mydriatic bases mentioned, almost all the other alkaloids dissolved in 0.5 N-sulphuric acid give precipitates with saturated bromine water and bromo-potassium bromide With the single exception of the caffeine precipitate, all the other precipitates of these alkaloids are amorphous Macroscopically they are either milky or flocculent in appearance, or they clump together in the manner of resins. Under the microscope, however, they do not consist of doubly refracting droplets

7. Physiological Test.—The action of atropine in causing enlargement of the pupil can be employed for the recognition of this alkaloid, for this enlargement of the pupil is distinct in the human or the cat's eye with one drop of an atropine solution 1:130,000. To make this test, dissolve the residue from the ether extract of the aqueous alkaline solution in 4-5 drops of extremely dilute sulphuric acid and introduce a drop of this solution into the conjunctival sac of one eye of a cat or dog, comparing the width of the two pupils. The eye of the rabbit is not suitable for this test.

Individual substances exerting mydriatic action upon the eye are very different with regard to speed of reaction, duration and intensity. Atropine, for example, is stronger and much more persistent in its action than homatropine. Cocaine enlarges the pupil but in much more concentrated solution than atropine and hyoscyamine and at the same time causes anaesthesia The action of l-hyoscy-

¹ See "Micro-sublimation" in Chapter V, page 471 of this book.

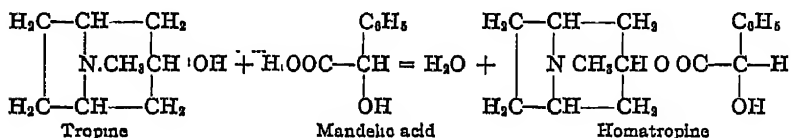
amine is nearly twice as strong as that of atropine and 12-18 times stronger than that of d-hyoscyamine. According to Joachimoglu,¹ scopolamine in the eye of the cat is about 10 times more active than atropine. Even 0.0002 mg. of atropine is said to produce mydriatic action in the human or cat's eye (Feddersen).

Notes.—Ptomaines exhibiting pronounced mydriatic action have repeatedly been found in putrefied flesh of fish. These extraordinarily toxic ptom-atropines are regarded by different investigators as the cause of poisoning from meat and especially from fish.

8. Gold Chloride Test.—The alkaloids possessing mydriatic properties may be distinguished from one another by preparing and characterizing their gold chloride double salts which crystallize well. This subject is more fully treated among the reactions of scopolamine (see page 185).

HOMATROPINE

Homatropine, $C_{15}H_{21}NO_3$, is the tropyl ester of phenyl-glycolic or mandelic acid. The hydrochloride of this base is obtained by heating tropine mandelate for several days in the water-bath with dilute hydrochloric acid, the latter acting as a dehydrating agent. The product of this reaction is then made alkaline with potassium carbonate and the free homatropine base precipitated as an oil is extracted with chloroform:



Distillation of the solvent leaves homatropine as an oil that crystallizes with difficulty. The free base forms colorless, hygroscopic prisms melting at 93.5-98.5°. On the other hand, homatropine hydrobromide, $C_{15}H_{21}NO_3 \cdot \text{HBr}$, employed in medicine, crystallizes very easily from water, forming wart-like clusters that dissolve readily in water and with difficulty in alcohol.

Detection.—Pure homatropine differs from atropine and hyoscyamine in not giving Vitali's test. The residue obtained by evaporating upon the water-bath even 0.01 gram of homatropine hydrobromide with fuming nitric acid has only a faint yellowish

¹ Joachimoglu. Berliner klinische Wochenschr. 52 (1915), 911.

color.¹ When cooled and treated with alcoholic potassium hydroxide solution, this residue acquires a yellow-red to red-yellow color.

If the odor test is made in the manner described for atropine, that is, without an oxidizing agent, homatropine gives an odor like that of benzaldehyde. Therefore it is easy to distinguish tropic acid derivatives by an odor like that of a flower or honey from mandelic acid derivatives, such as homatropine, by the benzaldehyde odor.

Homatropine is a strong base and consequently like atropine and hyoscyamine gives Gerrard's test. It also gives the test with phenolphthalein paper. Although giving the test with perhydrol-sulphuric acid, according to Wasicky homatropine does not respond to the test with p-dimethyl-amino-benzaldehyde and sulphuric acid. This serves to differentiate it from atropine, hyoscyamine and scopolamine.

With regard to its physiological properties, the action of homatropine upon the pupil of the eye is about as strong as that of atropine. The effect, however, disappears much more quickly, that is, in 12-24 hours, whereas the action of atropine may persist for 8 days. Consequently when it is a matter of producing enlargement of the pupil and having the action soon over, homatropine is to be preferred to atropine, because it affects accommodation slightly and only for a short time.

SCOPOLAMINE

Laevo-scopolamine, $C_{17}H_{21}NO_4 \cdot H_2O$, remains in the mother-liquors in the preparation of hyoscyamine and atropine from henbane seeds and those of *Datura Stramonium*. Larger quantities of scopolamine occur in the leaves of certain species of *Duboisia* and smaller quantities together with inactive scopolamine in the root of *Scopolia japonica*, *Scopolia atropoides* and other species of *Scopolia*. The root of *Atropa Belladonna* is said to contain very small quantities of scopolamine. (E. Schmidt.)

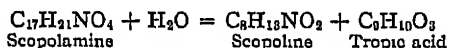
Properties.—Laevo-scopolamine crystallizes only with difficulty. It is deposited from a chloroform solution, when the solvent is distilled, as a viscous syrup. With some difficulty by means of ether this can finally be made to give colorless crystals melting at 59°. If crystallized scopolamine is kept over sulphuric acid, it changes to an amorphous, colorless, transparent mass. It dissolves rather easily in water, at least more easily than atropine. In alcohol, ether and chloroform it is freely soluble. The alcoholic solution of natural

¹ According to R. Eder (loc. cit.) the residue is colored distinctly yellow, if somewhat larger quantities of pure homatropine are present.

scopolamine is laevo-rotatory. If a little sodium hydroxide solution is added to this alcoholic solution, scopolamine gradually changes into optically inactive scopolamine. The latter probably bears the same relation to the natural laevo-rotatory base that inactive atropine does to active hyoscyamine.

Constitution of Scopolamine and Scopoline

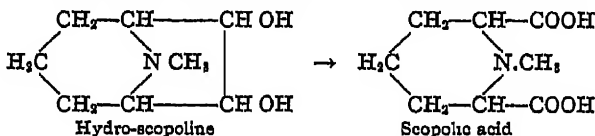
A warm solution of barium hydroxide brings about the hydrolytic cleavage of scopolamine into the base scopoline and the nitrogen-free constituent tropic acid:



When boiled for a longer time with considerable barium hydroxide solution, scopolamine undergoes cleavage into scopoline and atropic acid. Tropic acid, the first product of hydrolytic cleavage, loses a molecule of water and passes into atropic acid (E. Schmidt)

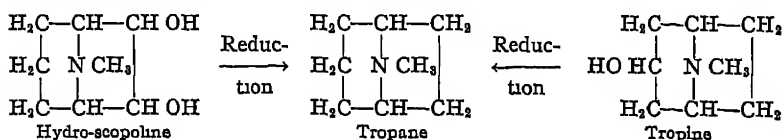


The constitution of tropic and atropic acid has been known for a long time. For some time it has been known, especially as a result of the researches of Schmidt, that one of the two oxygen atoms of scopoline belongs to a hydroxyl-group, whereas the other is in an ether-like union. Moreover a methyl-group is attached to nitrogen, since nor-scopoline is formed as the result of the cleavage of a methyl-group from scopoline through oxidation with permanganate, chromic acid, as well as through the action of bromine. By methylation of nor-scopoline it is possible to obtain scopoline again. Consequently the formula of scopoline may be resolved into $\text{C}_7\text{H}_9(\text{NCH}_3)(\text{OH})(\text{O} <)$. Within recent years the researches of Hess,¹ carried out with great thoroughness, have explained the constitution of scopoline and scopolamine. By warming scopoline with a solution of hydrobromic acid in glacial acetic acid, Hess obtained an excellent yield of an addition-product of both substances, hydro-scopoline bromide, from which may be obtained by means of nascent hydrogen, that is, with zinc dust and sulphuric acid, at room-temperature or with zinc dust and glacial acetic acid at about 110°, dihydro-scopoline, $\text{C}_8\text{H}_{13}\text{NO}_3$. The latter, briefly called hydro-scopoline by Hess, can be oxidized by chromic-sulphuric acid upon the water-bath to the dibasic scopolic acid, an isomer of tropic acid but entirely different from it in properties. Scopolic acid is identical with N-methyl-piperidine- α , α -dicarboxylic acid obtained synthetically by Hess. This reaction can be understood on the basis of the following formula of hydro-scopoline.



¹ K. Hess: Degradation of Scopoline I. (with A. Suchier). Scopoline \rightarrow Hydroscopoline \rightarrow Scopolic Acid. Ber. d. Deutsch. chem. Ges. 48 (1915), 2057. II. Communication, Ibid. 49 (1916), 2337, 2745. III. Communication, Ibid. 51 (1918), 1007. IV. Communication, Ibid. 52 (1919), 1947.

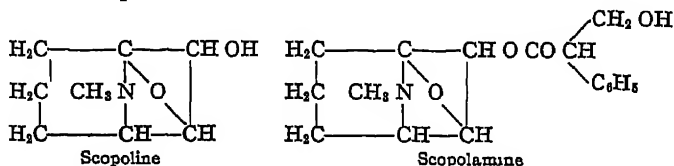
¹As a result of these experiments, Hess has furnished clear proof that scopoline, and consequently scopolamine, are alkaloids of the piperidine group and that the C-atoms in question are attached on both sides to the nitrogen atom in the α -position. The conversion of hydro-scopoline into an oxygen-free base may be accomplished by heating it with hydriodic acid and considerable phosphonium iodide for several hours at 200°. In this manner the oxygen-free derivative of tropine, tropane, is obtained in about 25 per cent yield.



Therefore hydro-scopoline is dioxy-tropane.

The constitution of scopoline is explained by these experimental results and its membership in the tropic group of alkaloids is clearly established.

Upon the basis of results obtained by the degradation of scopoline according to Hofmann's method, Hess has proposed the following structural formulae for scopoline and scopolamine.



Detection of Scopolamine

The mydriatic action of scopolamine is stronger than that of atropine and hyoscyamine. According to Joachimoglu,¹ scopolamine in the cat's eye is about 10 times more active than atropine. Scopolamine gives Guliemo's odor test. As in the case of all tropic acid derivatives, when heated in the manner described for atropine, it gives a characteristic odor of black-thorn blossoms and honey. Since scopolamine is a weaker base than atropine and homatropine, it does not give Gerrard's test with mercuric chloride, nor does it color phenol-phthalein paper (see Atropine, page 179). On the other hand, it gives both of the tests recommended by Wasicky for mydriatic alkaloids. Toward the two bromine reagents of Eder, scopolamine differs from the other mydriatics in producing precipitates, consisting only of amorphous droplets that crystallize but seldom and irregularly. Therefore a test should always be made with pure scopolamine as a check. If the available material is

¹ Berliner klinische Wochenschr. 52 (1915), 911

sufficient, the several mydriatic bases may be differentiated by preparing and identifying their gold chloride double salts, for these crystallize well, are difficultly soluble in cold water and have also a fairly sharp melting-point. To prepare these compounds, add a slight excess of chlor-auric acid solution to the alkaloid in question, for example, the residue from the ether solution, dissolved in dilute hydrochloric acid, and bring the precipitate into solution by warming. As the solution cools, the particular chloraurate will crystallize. The three most important compounds to be considered are:

Atropine Chlor-aurate.—This salt has the formula $C_{17}H_{23}NO_3 \cdot HCl \cdot AuCl_3$. At first it is oily but gradually solidifies. The crystals are dull and melt at $135-137^\circ$.

Hyoscyamine Chlor-aurate.—This salt has the formula $C_{17}H_{23}NO_3 \cdot HCl \cdot AuCl_3$. It crystallizes in gold-yellow leaflets that are highly lustrous and melt at $160-162^\circ$.

Scopolamine Chlor-aurate.—This salt has the formula $C_{17}H_{21}NO_4 \cdot HCl \cdot AuCl_3$. It crystallizes from hot water in broad yellow prisms that are difficultly soluble, well-formed and lustrous. They melt at $210-214^\circ$. Frequently these crystals have more of a feathery appearance under the microscope.

Combination of Morphine with Scopolamine.—This combination, frequently employed at the present time by surgeons, greatly increases the narcotic effect of anaesthetics taken by inhalation, making it possible to conduct and maintain narcosis with a considerably lower concentration of anaesthetic in the air inhaled. This combination alone makes it possible to bring about a condition in which there is freedom from pain and consciousness is dulled so that even major operations can be carried on painlessly. From many quarters the "Morphine-Scopolamine-Narcosis" according to Schneiderlin-Korff, has been recommended even as a substitute for anaesthesia by inhalation.

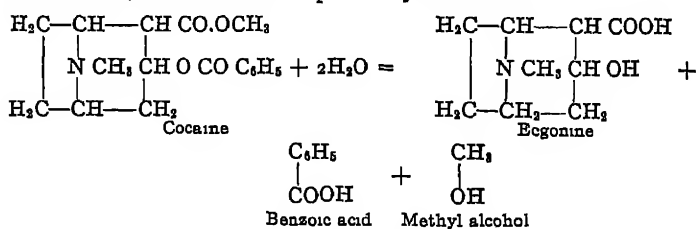
COCAINE

Cocaine, $C_{17}H_{21}NO_4$, occurs to the extent of 0.2-0.8 per cent in coca leaves, the leaves of the South American shrub, *Erythroxylon Coca*. Other coca species, such as those of Java and the East Indies, also probably contain cocaine. Among the accompanying alkaloids, occurring in coca leaves from different sources, may be mentioned: cinnamyl-cocaine, $C_{19}H_{23}NO_4$; benzoyl-ecgonine, $C_{16}H_{19}NO_4 \cdot 4H_2O$, cinnamyl-ecgonine, $C_{18}H_{21}NO_4$; tropa-cocaine, or benzoyl-pseudo-tropeine, $C_{16}H_{19}NO_2$; truxilline, $C_{19}H_{23}NO_4$; and hygrine, $C_8H_{15}NO$.

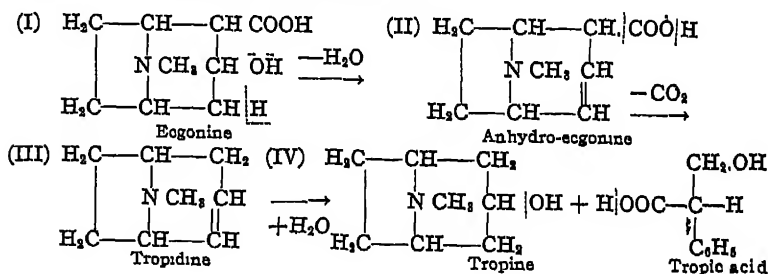
Properties.—Cocaine crystallizes best from hot alcohol or petroleum ether and is obtained in large, colorless, monoclinic prisms melting at 98° . Its solutions

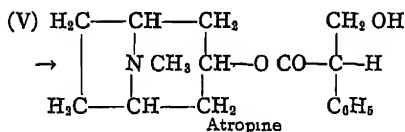
are laevo-rotatory and have a strong alkaline reaction. It has a faintly bitter taste producing upon the tongue temporary anaesthesia. Cocaine is difficultly soluble in water (1:700) but dissolves easily in alcohol, ether, benzene, chloroform and acetic ether. Dilute acids dissolve cocaine and usually form well-crystallized salts. The fixed alkalies, ammonia and alkaline carbonates precipitate the free base from solutions of its salts.

Constitution.—Cocaine is a monacid base, since it forms salts with one equivalent of acid. The most important cocaine salt is the hydrochloride, used in medicine and having the composition, $C_{17}H_{21}NO_4 \cdot HCl$. The tertiary nature of this alkaloid is shown by the fact that at 100° it combines with methyl iodide, forming the well-crystallized cocaine iodo-methylate, $C_{17}H_{21}NO_4 \cdot CH_3I$. It contains a methyl-group attached to nitrogen, that is, the group NCH_3 , because methylamine is split off when it is distilled with barium hydroxide. Moreover cocaine is the methyl ester of an acid and at the same time the benzoic acid ester of an alcohol, for when boiled with water it is decomposed into methyl alcohol and benzoyl-ecgonine. If mineral acids, barium hydroxide or alkalies are substituted for water, hydrolytic cleavage is carried farther with decomposition of benzoyl-ecgonine. In this way ecgonine, benzoic acid and methyl alcohol are obtained. The following equation expresses this cleavage on the basis of the structural formula of cocaine as proved by Willstätter



Ecgonine (I), the constitution of which has been clearly determined, loses water when heated with phosphorus oxychloride giving anhydro-ecgonine (II). The latter loses carbon dioxide, when heated at 280° with fuming hydrochloric acid, and yields tropidine (III). If this compound is boiled with sodium hydroxide solution, it adds a molecule of water and is converted into the basic cleavage-product of atropine, that is, tropine (IV). Under the influence of dilute hydrochloric acid, tropine and tropic acid condense with formation of atropine (V). Consequently it is possible by synthetic methods to convert the alkaloid cocaine into the solanaceous alkaloid atropine.





Physiological Properties and Toxic Action.—Cocaine is rapidly absorbed from all mucous surfaces. It has a two-fold action, that is, central and peripheral.

Even in small doses it exerts a central action, causing stimulation and increasing the efficiency of the brain and spinal cord (Kobert). Upon this property depends its use as a tonic and in producing exhilaration, as in the case of chewing coca leaves. After larger doses, this stimulation gives way to excitement and convulsions, followed finally by paralysis. Peripheral action, also local and produced by painting a solution of the alkaloid upon mucous surfaces and injecting it into other parts of the body, consists in deadening the sensitiveness of the sensory nerve endings.

Symptoms of cocaine poisoning that appear are dryness of the throat and nose, difficulty in swallowing, nausea, emesis, burning pain in the stomach, attacks of colic or gastric spasms, and loss of appetite. Additional symptoms that may be mentioned are loss of smell and taste, impairment of the sense of hearing and smell, enlargement and rigidity of the pupils, and lowering of the power of accommodation. Larger doses may give rise to high spirits, a condition akin to drunkenness, hallucinations, followed by depression with melancholia, a state of anxiety, and ideas of persecution. There is quickening of the pulse, derangement of the heart, irregular respiration, and even paralysis of respiration. Kunkel considers 1/2 gram as the smallest lethal dose of cocaine.

Elimination.—According to experiments made by Wiechowski,¹ dogs eliminate by way of the kidneys 10–12 per cent. of cocaine unchanged, whereas rabbits do not eliminate this alkaloid in this manner. Similar experiments with atropine have shown that dogs eliminate 17–57 per cent. of this alkaloid in the urine. The characteristic decomposition products of these two alkaloids (ecgonine from cocaine and tropine from atropine) could not be found in the urine of the experimental animals. The behavior of these two alkaloids in the animal body is analogous in that both undergo profound decomposition. After rather large doses, undecomposed cocaine is said to appear in human blood, organs and urine. According to Proelss,² cocaine can be detected in cadaveric material after 14 days at the maximum; also ecgonine is said to be rapidly separated in the living organism. Further details with regard to these matters are given below.

Detection of Cocaine

Ether, chloroform or benzene will extract cocaine from an alkaline aqueous solution. Provided the examination is confined to cocaine,

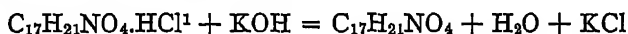
¹ W. Wiechowski: Fate of Cocaine and Atropine in the Animal Body. *Arch. f. exper. Pathol. u. Pharmacol.* 46 (1901), 155.

² H. Proelss: Contributions to the Detection of Alkaloids, Glucosides and Bitter Principles in Forensic-Chemical Investigations. *Apotheker-Zeitung* 16 (1901), 288, 306, 316, 325.

and there is no need of considering morphine, render the aqueous tartaric acid solution alkaline not with sodium hydroxide solution but with sodium carbonate, or better sodium bicarbonate, for cocaine is an ester and is easily decomposed by caustic alkalies. Most of the alkaloidal reagents will precipitate cocaine even from very dilute solutions of its salts. The reagents especially sensitive are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, phospho-molybdic acid, phospho-tungstic acid, gold chloride, plantinic chloride and picric acid. Pure concentrated sulphuric and nitric acid, as well as Erdmann's Froehde's and Mandelin's reagent, dissolve cocaine without color. This behavior differentiates cocaine from various other alkaloids. Cocaine picrate melts at 165-166°

Special Reactions

1. **Precipitation Test.**—If 1-2 drops of potassium hydroxide solution are added to an aqueous solution of a cocaine salt not too dilute, it will become milky. First oil-drops and later fine crystalline needles of the free base separate:



In applying this test to the residue from the ether extract of the aqueous alkaline solution, dissolve it first in a few drops of dilute hydrochloric acid and add potassium hydroxide solution drop by drop in excess, cooling well by setting in ice. Special care must be taken to have the alkaloid pure enough so that, after filtration, washing and drying, its melting-point (98°) can be determined.

Obviously, precipitation of cocaine from solutions of its salts by potassium hydroxide solution is not characteristic, for under these conditions most alkaloids are similarly precipitated.

2. **Potassium Permanganate Test.**—Add saturated potassium permanganate solution drop by drop to a concentrated aqueous solution of a cocaine salt. A violet, crystalline precipitate of cocaine permanganate will appear. In applying this test to the residue from the ether solution of II B, dissolve it first in a few drops of dilute hydrochloric acid, evaporate the solution upon the water-bath, and

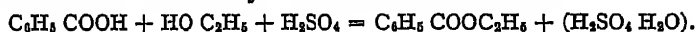
¹ Cocaine hydrochloride crystallizes from a concentrated aqueous solution in fine prisms containing 2 molecules of water of crystallization which it easily loses. This salt crystallized from alcohol is anhydrous and is the official *Cocainum Hydrochloricum* having the composition $C_{17}H_{21}NO_4.HCl$.

test the solution of the dry residue in water with potassium permanganate solution (1:100).

3. Chromic Acid Test.—Add 5 per cent. chromic acid solution, or potassium dichromate solution of corresponding concentration, to a solution of a cocaine salt not too dilute. Each drop will produce a precipitate that will immediately disappear upon shaking the solution. Then add to the clear solution about 1 cc. of concentrated hydrochloric acid. This will produce a more or less crystalline, orange precipitate.

Only when the quantity of cocaine for examination is not too small, do tests 1-3 give a positive result admitting of no doubt. They are applicable therefore for the identification of cocaine in pharmaceutical examinations rather than for the purposes of forensic chemistry.

4. Detection of Benzoyl-group.—This test requires at least 0.2 gram of cocaine. Warm the cocaine for a few minutes in a small test-tube with 2 cc. of concentrated sulphuric acid in a boiling water-bath. Cool and dilute with water, adding it drop by drop and all the while keeping the mixture cold. A white crystalline precipitate of benzoic acid will appear. Filter, wash with a little ice-water and dry this precipitate. Benzoic acid may be identified by sublimation, or by determining its melting-point (120°), provided the quantity is sufficient. Benzoic acid may also be extracted with ether. Evaporate the ether solution and warm the residue with 1 cc. of absolute alcohol and the same quantity of concentrated sulphuric acid. The characteristic odor of ethyl benzoate will be detected:



In doubtful cases make a similar test with pure benzoic acid as a check.

5. Iodic Acid Test.—Add a few drops of concentrated sulphuric acid to a trace of cocaine and then a small particle of potassium iodate free from iodide, or iodic acid. No color appears in the cold but, if this mixture is heated in a porcelain dish over a small flame until vapors of sulphuric acid come off, and just a little longer, there appear in succession and together brown, olive-green, blue and violet shades of color which issue from the particle of iodate and immediately disappear. Finally vapors of iodine are also given off. According to Gadamer, this play of colors is really due to the presence of the benzoyl-group in the cocaine molecule, for pure benzoic acid gives it especially clearly. By this iodic acid test it is said to be possible to detect even 0.05 mg. of cocaine. But other alkaloids also give

intense colorations with sulphuric acid and potassium iodate and usually even in the cold. Under the same conditions ecgonine gives cherry to purple-red colors that finally become dirty violet.

6. Physiological Test.—Dissolve the given material (the residue from the ether extract of the aqueous alkaline solution) in a few drops of dilute hydrochloric acid and evaporate the solution to dryness upon the water-bath. Dissolve the residue in a little pure water and place a drop of this solution upon the tongue. If cocaine is present, it will produce temporary anaesthesia of the tongue.

R. Kobert ("Intoxikationen") has found rather small frogs sufficiently sensitive for the physiological identification of cocaine. The effects to be observed are dilatation and fixedness of the pupil, enlargement of the palpebral fissure and also stimulation of the nervous system. Administer about the same quantity of cocaine hydrochloride to another animal as a check.

The fact that cocaine does not give Vital's test differentiates it from the alkaloids atropine, hyoscyamine and scopolamine which are also mydriatic in action. Moreover, since cocaine is easily soluble in petroleum ether, whereas atropine is not, this solvent may be used in extracting the former alkaloid.

Detection of Cocaine and Novocaine in the Cadaver

According to different statements in the literature, it is said that cocaine may be detected in the cadaver even 14 days after death. As the result of an examination for the purpose of determining whether a solution of cocaine instead of novocaine had been injected into two female patients, who had died with symptoms of cocaine poisoning, Popp¹ was unable to establish the presence of either cocaine or novocaine in the organs received 5 days after death. In connection with this investigation Popp together with Mannich and Ellinger conducted experiments upon rabbits, injecting into them varying quantities of cocaine and novocaine. The animals were killed at different intervals after injection and the extracts from their organs tested for cocaine by their anaesthetizing action and by the potassium permanganate and iodic acid test. Only in a single instance was cocaine detected at the place of injection 3 hours after death. Novocaine in doses of 100 mg. for each animal was also detected only at the place of injection but even when putrefaction was beginning. Separation of cocaine and novocaine, carried out according to the method of Kircher-Hoechst (see below), succeeded only with quantities of at least 2 mg. All the older statements with regard to the detection of cocaine in the cadaver after longer periods should be viewed with scepticism. Cocaine probably belongs to those poisons which

¹ G. Popp: Detection of Cocaine and Novocaine in Cadaveric Material. Abstract of an address delivered at the principal meeting of the society of independent public chemists in the *Chemiker-Zeitung* 1922.

very seldom be detected in the cadaver. Apparently the cleavage of the thoxy and benzoyl-group from the cocaine molecule in the animal organism is accomplished with the greatest ease (Heiduschka).

In the detection of novocaine, use may be made of the primary, aromatic amino-group which can be diazotized and then coupled with formation of an azo-dyestuff. Its detection in urine is usually attempted in this manner

Detection of Cocaine in Novocaine

(Kircher¹)

Acidify the given novocaine-cocaine solution with dilute hydrochloric acid, dilute and add drop by drop 0.5 per cent sodium nitrite solution until after standing 15 minutes it still turns starch-iodide paper blue. Now add to the diazotized solution enough filtered alumol solution (aluminum salt of β -naphthol-sulphonic acid) rendered alkaline with sodium carbonate, transfer the deep blue mixture to a separating funnel, and repeatedly extract with sufficient quantities of benzene. The latter takes up cocaine unchanged by diazotization together with small quantities of dyestuff. To remove the dyestuff, repeatedly wash the cocaine-benzene solution with small quantities of water and then extract with an excess of dilute hydrochloric acid. Evaporate the aqueous solution of cocaine hydrochloride thus obtained upon the water-bath to the point of crystallization and test these crystals as described on page 188 for cocaine.

PHYSOSTIGMINE

Physostigmine, or eserine, $C_{16}H_{21}N_3O_2$, occurs in the cotyledons of the seeds of *Physostigma venenosum* to the extent of about 0.1 per cent together with eseridine, $C_{16}H_{23}N_3O_2$. The former has been detected also in pseudo-Calabar beans, Calab-nuts.

Properties.—Physostigmine is obtained in large rhombic crystals, melting at 5° , by spontaneous evaporation of its benzene solution. Though only slightly soluble in cold water, it dissolves freely in alcohol, ether, benzene and chloroform. Physostigmine solutions are strongly alkaline, nearly tasteless and laevo-rotatory. Physostigmine is heated for some time at 100° , it turns red and then gives red solutions with acids. This alkaloid, notwithstanding the presence of three nitrogen atoms, is only a monacid base forming salts with one equivalent of acid and easily undergo decomposition and crystallize with difficulty. Physostigmine sulphate, $(C_{16}H_{21}N_3O_2)_2 \cdot H_2SO_4$, and physostigmine salicylate, $C_{16}H_{21}N_3O_2 \cdot H_4(OH)COOH$, the salts of this alkaloid that are most stable and most easily obtained in crystalline condition, are used as myotics in ophthalmology and also as purgatives. Caustic alkalies, sodium carbonate and bicarbonate set physostigmine free from its salts, without causing a precipitate when the solution is quite dilute, so that the free base can be extracted by ether or chloroform. Since

¹ Private information from Dr. Kircher of the laboratory for pharmaceutical investigation of the Color Works at Höchst on the Main, kindly submitted in a private communication of Dr. G. Popp of Frankfurt on the Main.

physostigmine forms an iodo-methylate, $C_{15}H_{21}N_3O_2 \cdot CH_3I$, with methyl iodide, it is a tertiary base, and since it gives methyl amine together with other bases when distilled with potassium hydroxide, the group NCH_3 must be present. The latter gives physostigmine its basic properties. Moreover it contains a hydroxyl-group.

Physiological Properties.—Physostigmine exhibits central and peripheral action, the former consisting of stimulation of the brain and spinal cord with subsequent paralysis. The peripheral action of physostigmine is strikingly like that of pilocarpine and muscarine with respect to the eye, salivary glands, gastro-intestinal tract, etc. As a result of this action, contraction of the pupils, which may persist for 10–24 hours, occurs, glandular secretion is heightened, and the action of the heart and intestines is increased. Consequently its action is just the reverse of that of the mydriatic alkaloids. This explains how atropine in not too small doses can at least partially neutralize the effects of physostigmine. Internal administration of physostigmine does not always cause contraction of the pupils (myosis) either in man or horses. Some of the usual symptoms that may be mentioned are watering of the eyes, flow of saliva, severe sweating, diarrhoea, weakness of the heart, slowing of the pulse, increase of blood-pressure, uneasiness, and sometimes maniacal attacks. Death ensues from paralysis of respiration.

Physostigmine is easily absorbed and rather quickly eliminated in the urine, bile, saliva and milk. Without doubt a large part of the alkaloid is destroyed in the body. Detection of physostigmine in the urine is frequently possible.

A noteworthy result is the effect of magnesium salts in counteracting trembling of the limbs (tremor) occasioned by physostigmine. On the contrary they are without influence upon contraction of the pupils. Consequently magnesium salts are useful in certain cases as an antidote for eserine (Joseph¹).

Detection of Physostigmine

Since physostigmine is easily decomposed by strong bases, the aqueous tartaric acid solution in the Stas-Otto process should first be covered with ether, then rendered alkaline with sodium hydroxide solution, and at once shaken vigorously. In this way physostigmine, if present, is dissolved by ether and removed from the destructive action of caustic alkali. Provided there is no reason to include morphine in the examination, sodium bicarbonate may be substituted for sodium hydroxide. When acid and alkaline solutions of physostigmine are warmed in contact with air, or even exposed to light, they turn red. This is due to formation of rubreserine. Because of the ease with which physostigmine undergoes decomposition, material such as vomitus, consisting of gastric as well as intestinal contents, liver, bile, blood and urine, should be acidified with tartaric acid and

¹D. R. Joseph: Inhibiting Influence of Magnesium upon Certain Toxic Effects of Eserine. *Amer. Journ. Physiol.* 23 (1909), 215.

extracted with alcohol in the dark at room temperature. Alcohol in the filtered extract, protected from light, should be distilled *in vacuo*, the residue filtered, the filtrate rendered alkaline with sodium bicarbonate and extracted with ether. To purify the residue from this ether extract, dissolve it in cold water containing tartaric acid, filter the solution, render alkaline with sodium bicarbonate, and again extract with ether. The residue from this ether solution should be tested for physostigmine.

Special Reactions

1. Concentrated nitric acid dissolves physostigmine with yellow color. Concentrated sulphuric acid dissolves it, according to the degree of purity, colorless or with yellow color.

2. **Ammonia Test.**—Dissolve physostigmine in water with a trace of sulphuric acid, then add a few drops of ammonia, and evaporate to dryness upon the water-bath. First a red color appears but the residue is blue. Should it show a tint of red, evaporate it once more with ammonia. The blue residue will dissolve in alcohol with a blue color. If acetic acid or mineral acid is added, the solution by transmitted light will appear litmus-violet, but by reflected light red, exhibiting at the same time a fine eosine-red fluorescence. If again rendered alkaline with ammonia, chloroform will dissolve the dyestuff with a green-blue color (Gadamer).

Gaubert's¹ Procedure.—If an aqueous physostigmine solution is allowed to stand for a month until it has taken on a deep blue color, addition of phthalic anhydride will produce blood-red fluorescence. This new substance may be obtained in dark blue crystals that dissolve in alcohol and ether, giving dark blue solutions that do not fluoresce. On the other hand, the aqueous solution appears nearly colorless by transmitted light but by reflected light exhibits brilliant blood-red fluorescence. According to Gaubert, this substance is more strongly fluorescent than any substance at present known.

3. **Rubreserine and Eserine Blue Test.**—If an aqueous solution of a physostigmine salt is shaken for some time in contact with air with an excess of potassium or sodium hydroxide solution, cleavage of carbon dioxide and methyl amine takes place with formation of the red dyestuff, rubreserine. This compound separates as red needles that become greenish blue upon further oxidation owing to

¹ P. Gaubert. A New Highly Fluorescent Substance obtained from Physostigmine. *Compt rend de l'Acad. des sciences* 149 (1909), 852.

formation of eserine blue. If barium hydroxide solution is substituted for alkaline hydroxide, a white precipitate first appears soon becoming red when shaken. Sometimes this change occurs even in the cold but invariably with heat

4. **Physiological Test.**—The marked action of physostigmine in causing contraction of the pupil is very characteristic. To make this test, dissolve the residue from the ether solution in water containing a trace of hydrochloric acid and introduce this solution into the conjunctival sac of a cat. Even 0.1 milligram of physostigmine will produce noticeable contraction.

PILOCARPINE

Pilocarpine, $C_{11}H_{16}N_2O_2$, together with iso-pilocarpine and probably also pilocarpidine, is a constituent of true jaborandum leaves, the leaves of *Pilocarpus pennatifolius*,¹ a member of the Rutaceae family, indigenous to Brazil. False jaborandum leaves, the leaves of *Piper reticulatum* and of other species of *Piper* and *Pilocarpus*, also appear to contain pilocarpine in varying quantities. Genuine hairy jaborandum leaves yield about 1 per cent. of pilocarpine

Properties.—The free base pilocarpine is usually obtained as a semi-liquid, viscous, non-volatile mass having an alkaline reaction. It dissolves but slightly in water, is freely soluble in alcohol, ether and chloroform, but insoluble in benzene. Solutions of pilocarpine and its salts are dextro-rotatory. This alkaloid is a strong base neutralizing acids and forming salts that are usually crystalline. Caustic alkalis, added to concentrated solutions of pilocarpine salts, precipitate the free base which redissolves in excess of precipitant. Solutions of sodium hydroxide, or sodium ethylate, cause molecular rearrangement of pilocarpine. This reaction runs more smoothly, if pilocarpine hydrochloride is heated for half an hour at 200° . The product of this change is iso-pilocarpine, isomeric and very likely stereo-isomeric with pilocarpine. Both isomeric pilocarpines differ in melting-points, solubilities and particularly in specific rotation. Iso-pilocarpine is less dextro-rotatory than pilocarpine and crystallizes in deliquescent prisms easily soluble in water and alcohol. The salts of the two bases also show similar differences:

Pilocarpine nitrate. $C_{11}H_{16}N_2O_2 \cdot HNO_3$; mpt 178° ; $[\alpha]_D = +85.90^\circ$.

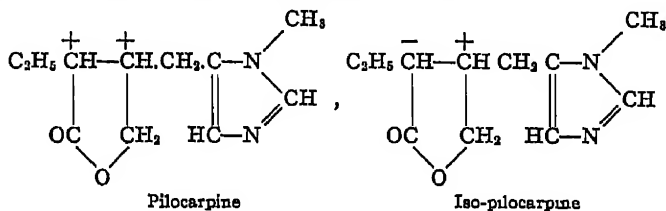
Iso-pilocarpine nitrate. $C_{11}H_{16}N_2O_2 \cdot HNO_3$; mpt 159° ; $[\alpha]_D = +35.68^\circ$.

Jowett² has succeeded in converting iso-pilocarpine into pilocarpine by the same reagent used in converting pilocarpine into iso-pilocarpine. Pure iso-

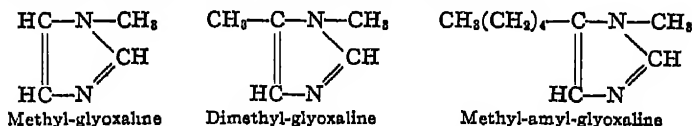
¹ According to the researches of H. A. D. Jowett, jaborine, described as another alkaloid in jaborandum leaves, is a mixture of iso-pilocarpine, pilocarpidine, a little pilocarpine and coloring-matter.

² H. A. D. Jowett: Constitution of Pilocarpine, Part I. Conversion of Iso-pilocarpine into Pilocarpine. Proc. Chem. Soc. 21 (1905), 172; Journ. Chem. Soc. London 87 (1905), 794.

pilocarpine, heated with alcoholic potassium hydroxide, gives a mixture of unaltered iso-pilocarpine and pilocarpine. The identity of the latter with pure pilocarpine was established by preparing the hydrochloride, melting at 201° , $[\alpha]_D = +92.8^{\circ}$; and nitrate, melting at $177-178^{\circ}$. This reciprocal conversion of one alkaloid into the other strongly supports the idea of the stereo-isomerism of pilocarpine and iso-pilocarpine. Pilocarpine combines with one equivalent of acid, forming crystalline salts that are usually easily soluble in water and alcohol. Consequently pilocarpine is a monacid base. Pilocarpine hydrochloride, $C_{11}H_{15}N_2O_2 \cdot HCl$, usually in the form of laminated crystals, having a faint acid reaction and bitter taste, is used in medicine. It abstracts moisture from the air and therefore is deliquescent. Caustic alkalies and barium hydroxide solution cause temporary precipitation of the free base only from concentrated solutions of pilocarpine salts, for it redissolves in excess of precipitant forming salts of pilocarpic acid, $C_{11}H_{13}N_2O_3$. Mineral acids liberate from these salts pilocarpic acid which at once loses water and passes into its inner anhydride, pilocarpine. On the basis of this behavior pilocarpine is a lactone. By distillation with soda lime, Jowett obtained methyl-glyoxaline, dimethyl-glyoxaline and methyl-amyl-glyoxaline. According to this behavior, pilocarpine is a derivative of glyoxaline. In view of these facts, therefore, pilocarpine and iso-pilocarpine must be tertiary bases containing in the molecule a lactone and a glyoxaline ring. This confirms the structural formula first proposed by A. Pinner. Jowett explains the stereo-isomerism in the following manner:



The glyoxaline bases obtained by means of soda lime are:



The unsaturated character of the glyoxaline ring probably accounts for the ease with which pilocarpine undergoes oxidation.

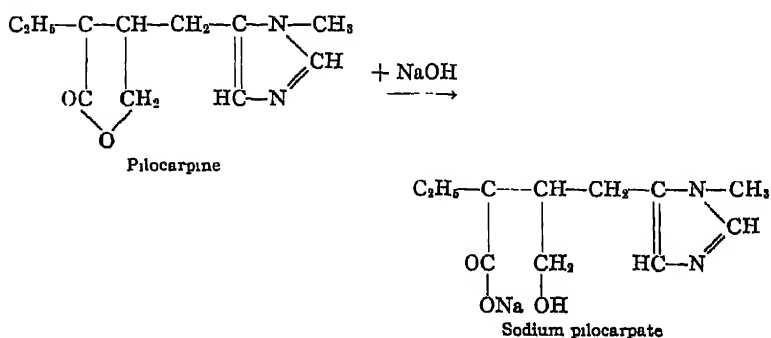
Physiological Action.—The effects of pilocarpine upon the animal organism very closely resemble those of nicotine except that in the case of pilocarpine peripheral effects preponderate. Pilocarpine in its action is antagonistic to atropine, for it increases the secretions, especially that of the saliva, sweat and of the intestinal mucosa. Secretions from all the other glands are increased, for example, from the lachrymal, bronchial and tracheal glands. Consequently pilocarpine is used in medicine to cause flow of saliva, perspiration, intestinal secretion and mucous secretion of the respiratory tract. The symptoms of pilocarpine poisoning mentioned by Kobert are. flow of saliva, sweating, weep-

ing, profuse secretion from the nose, emesis, diarrhoea, contraction of the pupils, indistinctness of vision, palpitation of the heart, retarded pulse, headache, dizziness, rattling in the trachea, and oedema of the lungs. There is copious formation of mucus in the lungs which may give rise to attacks of coughing and asphyxiation. Atropine is very useful as an antidote. Pilocarpine is easily absorbed and probably is quickly eliminated. In keeping with its chemical structure and on account of the ease with which it undergoes oxidation, it is probably decomposed in the human body, if not completely, at least in the main so that at most only traces of undecomposed pilocarpine can be detected in the urine and in the organs of the body.

Detection of Pilocarpine

Ether, chloroform or benzene extracts pilocarpine from aqueous solutions that have been rendered alkaline with ammonia, or better with sodium bicarbonate. Evaporation of these solutions leaves a thick, non-crystalline, alkaline syrup or varnish. The general reagents especially sensitive to pilocarpine are: iodo-potassium iodide, phospho-molybdic acid, phospho-tungstic acid and potassium bis-muthous iodide.

If the examination follows the usual procedure of the Stas-Otto process and the tartaric acid solution is rendered alkaline with sodium hydroxide solution, the lactone ring of pilocarpine, as soon as this base is set free, is broken and it passes into solution as sodium pilocarpate:



If the solution is acidified, the reaction is reversed. Free pilocarpic acid loses water and forms its lactone pilocarpine which remains in solution as a salt. If this acid solution is made alkaline with ammonia or sodium bicarbonate, free pilocarpine may be extracted with ether or chloroform and the residue from this extract examined by Helch's test or biologically.

Special Reactions

1. **Helch's¹ Test.**—Put a particle of potassium dichromate and 1 cc. of chloroform into a test-tube. Then add pilocarpine its or its solution, and 1 cc. of 3 per cent hydrogen peroxide. Shake for several minutes without interruption. The mixture yellow at first gradually darkens and in 5 minutes is dark brown. Depending upon the amount of pilocarpine, the chloroform is blue-violet, dark blue or indigo-blue, but the upper aqueous solution gradually fades. The chloroform is an intense blue from 0.01 gram of pilocarpine and blue-violet from 0.001 gram and less. Sometimes the color persists for a day. This reaction, however, is not confined exclusively to pilocarpine.

Notes—Even without hydrogen peroxide, 0.01 gram of apomorphine colors chloroform blue-violet. Strychnine gives a barely perceptible bluish tint that fades entirely within a few minutes. Antipyrine gives a blue color in chloroform only after acidification of the hydrogen peroxide. Salicyrine and mugraim behave in the same manner.

2. **Physiological Test.**—Dissolve the residue from the ether-chloroform solution in water containing a drop of hydrochloric acid. Evaporate this solution upon the water-bath to remove excess of hydrochloric acid and introduce the pure aqueous solution of the residue into the conjunctival sac of a cat's eye. If pilocarpine is present, it will cause contraction of the pupil. The human eye is also very sensitive to the action of this alkaloid. Muscarine, nicotine and physostigmine, as well as pilocarpine, cause contraction of the pupil, or myosis.

Thus far fatal poisoning from this alkaloid has not occurred and nothing is known with regard to the possibility of its detection in the cadaver.

CODEINE

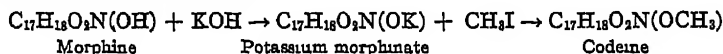
Codeine, $C_{18}H_{21}NO_3 \cdot H_2O$, occurs to the extent of 0.2–0.8 per cent. in almost all kinds of opium. It is the methyl ether of monacid phenol morphine. Eucodine² is a codeine derivative.

¹ H. Helch. *Reactions of Identification of Pilocarpine Hydrochloride*. Pharmacol. 35 (1902), 289, 39 (1906), 313.

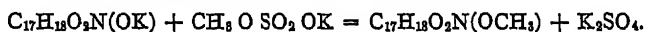
² Codeine bromo-methylate, $C_{17}H_{19}O_2(OCH_3)N \cdot CH_3Br$, obtained from anhydrous codeine and methyl bromide dissolved in chloroform, has been introduced into medicine under the name eucodine. It is said to be much less toxic than codeine, that is, convulsive action has been greatly diminished. Eucodine forms lustrous prisms having a bitter taste and also dissolving easily in water. Its solutions have a neutral reaction (distinction from codeine) and melts with decomposition at 261°.

Properties.—Codeine crystallizes from pure water, or from ether containing water, in colorless, transparent octahedrons that are often very large and contain 1 molecule of water. But from its solution in anhydrous ether or benzene it appears as smaller, rhombic crystals that are anhydrous, very lustrous and melt at 155° . The hydrated crystals lose crystal water at 100° . Codeine dissolves at 15° in 118 parts and at 100° in 15 parts of water, giving solutions that have a bitter taste, a strong alkaline reaction, and turn the plane of polarized light to the left. Codeine differs from most of the other alkaloids, especially from morphine, by its relatively high solubility in water. Hydrated codeine dissolves very readily in alcohol, ether, benzene and chloroform but very slightly in carbon disulphide. Codeine is as soluble in ammonia as in water but almost insoluble in sodium and potassium hydroxide solution. In this respect it differs from morphine which dissolves easily in solutions of caustic alkalis.

Preparation by Methylating Morphine.—1. Morphine is methylated when heated at about 60° in methyl alcohol solution with methyl iodide in presence of sodium or potassium hydroxide.



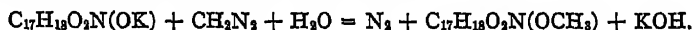
2 The same result may be accomplished by heating morphine, dissolved in a methyl alcohol solution of sodium or potassium hydroxide, with potassium or sodium methyl sulphate for several hours under a reflux.



3 Gently heat a solution of sodium morphinate in methyl alcohol with dimethyl sulphate. Sodium methyl sulphate will separate out but codeine will remain in solution.



4. Codeine is also formed as the result of the action of diazo-methane in the nascent condition upon potassium morphinate



In these reactions of synthesis, the hydrogen of the phenolic hydroxyl-group of morphine is replaced by methyl.

Constitution.—Codeine is a strong monacid base which completely neutralizes acids and usually forms crystalline salts. Codeine phosphate, $\text{C}_{17}\text{H}_{18}\text{O}_2(\text{OCH}_3)\text{-N H}_2\text{PO}_4\text{H}_2\text{O}$, separates upon addition of alcohol to a saturated solution of codeine in official phosphoric acid in short prisms, or as a white, crystalline powder. It has many uses in medicine. In water it dissolves with ease (1:3.2) but is only slightly soluble in alcohol. Codeine is a tertiary base, since it combines with methyl or ethyl iodide, when heated at 100° in presence of a little alcohol, forming the finely crystallized codeine iodo-methylate, $\text{C}_{17}\text{H}_{18}\text{O}_2(\text{OCH}_3)\text{-N.CH}_2\text{I}$, or codeine iodo-ethylate, $\text{C}_{17}\text{H}_{18}\text{O}_2(\text{OCH}_3)\text{N C}_2\text{H}_5\text{I}$, from which moist silver oxide sets free the unstable ammonium bases, codeine methyl hydroxide, $\text{C}_{17}\text{H}_{18}\text{O}_2(\text{OCH}_3)\text{N CH}_2\text{OH}$, and the corresponding ethyl derivative. When heated with potassium hydroxide, codeine gives off methyl amine, together with a little trimethylamine, proving that the codeine molecule contains the group $=\text{N CH}_3$.

Physiological Action.—The particular difference between codeine and morphine is that the narcotic action of the former upon the brain-centers is very much reduced but continues to affect the respiratory center. Within recent years use of codeine as a cough remedy has increased considerably. As a cough remedy, a dose of 0.03–0.06 gram of codeine phosphate acts upon man as effectively as 0.005–0.01 gram of morphine. Its toxicity is about 20 times less than that of morphine. Even large doses do not produce true narcosis but the symptoms observed are, restlessness, slight muscular twitching, as well as mydriasis, except in certain cases of idiosyncrasy. Codeine is less adapted for alleviating pain and as a sedative (Hans Meyer and R. Gottlieb). Doses of 0.8 gram of codeine gave rise to: trembling, muscular weakness, visual disturbances, myosis, feeling of dizziness, quickening of the pulse, and collapse (Lewin). In addition, marked dilatation of the pupils, persisting several days, flow of saliva, and very defective respiration appear (Kobert). Following subcutaneous injection, codeine may be found in the stomach, intestines, blood and liver. Experiments by Bouma¹ upon dogs showed in one case that 77.1–89.6 per cent of codeine, administered in daily injections of 0.2–0.4 gram of codeine phosphate over rather a long period, was eliminated unchanged in the urine and faeces together; and 81.1–83.1 per cent in a second case. Although the body, as the result of continued administration of codeine, does not acquire the power of decomposing this alkaloid, it gives no indication of becoming habituated to it. On the contrary, the fact was established that the experimental animals exhibited increased sensitiveness. Probably there is no codeinism. Elimination of codeine as such proceeds by way of the urine and faeces, but principally by the former. After subcutaneous injection, codeine was found in the stomach, intestines, blood and liver.

Detection of Codeine

Codeine can be extracted from the aqueous alkaline solution by ether, benzene and chloroform. This alkaloid appears in the ether residue usually as a non-crystalline varnish in which it may be detected by the following tests:

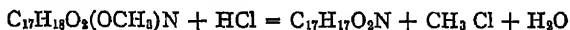
1. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves pure codeine without color. After several days standing in the cold, or at once after gentle heating, the solution becomes reddish to brownish violet. If the solution of codeine in concentrated sulphuric acid is heated upon a watch-glass to about 150°, that is, until white vapors appear, and cooled, it will give a deep red color with a drop of concentrated nitric acid. This test like that of Pellagri (3) is due to formation of apomorphine by heating the sulphuric acid solution of codeine and detection of this base with nitric acid:



¹ J. Bouma: Experiments upon Habituation to Codeine. Arch. f exper Path. u. Pharm. 50 (1903), 353.

2. Nitric Acid Test.—Concentrated nitric acid dissolves codeine with yellow color

3. Pellagri's Test.—Both codeine and morphine give this test. Dissolve codeine in 3-4 cc of concentrated hydrochloric acid and 3-4 drops of concentrated sulphuric acid. Evaporate this solution in a porcelain dish upon the water-bath, until the odor of hydrochloric acid is gone, and then allow it to stand about half an hour longer upon the boiling water-bath. Dissolve the dirty red or violet residue in about 3 cc of water, add a few drops of dilute hydrochloric acid and solid sodium bicarbonate to a faint alkaline reaction. Then add drop by drop, at first only 2 drops, dilute alcoholic iodine solution and shake well for several minutes after each addition. Presence of codeine is indicated by an emerald-green color that frequently is intensified by another drop of iodine solution. Shake in a test-tube with 2-3 cc of ether. The solvent will have a fine red color, whereas the aqueous solution will remain green. The following equation explains this test:



4. Oxidation Test.—Mix a little codeine upon a watch-glass with 4 times the quantity of finely powdered potassium arsenate (KH_2AsO_4). Add a few drops of concentrated sulphuric acid and then warm this mixture very gently over a small flame. The acid will have a deep blue color or, if the codeine is not quite pure, more of a bluish to reddish violet. If water or sodium hydroxide solution is added, the blue color will change to orange-yellow. Excess of potassium arsenate does not affect the test.

A trace of ferric chloride solution may be substituted for potassium arsenate as an oxidizing agent. Sulphuric acid containing 1 drop of ferric chloride solution to 10 cc of acid is prescribed by the German Pharmacopoeia to detect codeine in codeine phosphate.

5. Froehde's Test.—This reagent dissolves codeine with a yellowish color which soon changes to green and finally to deep blue. Gentle warming of the solution over a very small flame will hasten this change of color.

R. Mauch warms 2-3 drops of a chloral hydrate solution of codeine with 1 drop of Froehde's reagent. An intense blue color finally appears.

6. Marquis'¹ Test.—Concentrated sulphuric acid containing formalin dissolves codeine with a reddish violet color. This soon

¹ See preparation of reagents, page 642

changes to blue-violet which persists for quite a long time. The spectrum of this solution shows absorption of orange and yellow.

7. Mecke's¹ Test.—Concentrated sulphuric acid containing selenious acid dissolves codeine with blue color quickly changing to emerald-green and finally becoming permanent olive-green. Gentle heat will produce a steel-blue color.

8. Furfural² Test.—Dissolve codeine in a little concentrated sulphuric acid and warm very gently with a drop of cane-sugar solution which must not be in excess. This will produce a purple-red color. This test may also be made by mixing a drop of sugar solution with codeine, dissolved in about 5 drops of 50–60 per cent aqueous chloral hydrate solution, and then adding 1–2 cc of concentrated sulphuric acid as an under layer. A carmine-red ring will appear at the zone of contact. The color is quite permanent and increases in intensity upon standing. If the sulphuric acid and chloral hydrate are thoroughly mixed, the entire liquid will be red. After a time the shade of color will be more of a red-brown.

Of the general alkaloidal reagents, iodo-potassium iodide, potassium bismuthous iodide, potassium mercuric iodide, and phosphomolybdic acid precipitate codeine even from very dilute solutions. Tannic acid, picric acid, gold and platinum chloride on the other hand are not as delicate.

Differences between Codeine and Morphine.—Morphine is soluble in solutions of caustic alkalies, codeine is not. The latter can be completely extracted by ether from an aqueous alkaline solution, morphine cannot, or at most only in traces. Codeine does not set iodine free from iodic acid, nor give with a mixture of ferric chloride and potassium ferricyanide a blue color, or blue precipitate. Solutions of neutral codeine salts are not colored blue by ferric chloride.

A cadaveric codeine has been described in the literature but it failed to give Pellagrini's test, so characteristic of morphine and its derivatives. Therefore any possibility of confusing it with codeine is excluded.

NARCOTINE

Narcotine, $C_{22}H_{23}NO_7 = C_{10}H_{14}NO_4(OCH_3)_3$, is a component of opium, occurring to the extent of 4–8 per cent and principally as the free alkaloid. In smaller quantity it appears to be present also in the officinal *Capita papaveris*.

¹ See preparation of reagents, page 643.

² This test depends upon furfural formed by the action of concentrated sulphuric acid upon cane-sugar. Very dilute aqueous furfural solution (1:1000) may be substituted for cane-sugar. Excess of furfural unlike cane-sugar does not interfere with the test. Tr.

Properties.—Narcotine crystallizes from alcohol in shining prisms, or in tufts of long needles, that are nearly insoluble in cold and but slightly soluble in boiling water. It dissolves most easily in chloroform and boiling alcohol. From the latter solution it crystallizes almost completely when cold. At 15° narcotine dissolves in 170 parts of ether, 31 parts of acetic ether, and 22 parts of benzene. Solutions of narcotine are neither alkaline nor bitter. In these respects narcotine is very different from the other opium alkaloids, as well as from the various other plant bases. Narcotine combines with one equivalent of acid, forming salts that usually do not crystallize. Their aqueous solutions have an acid reaction and they are readily soluble in alcohol. Narcotine salts of the weaker acids are decomposed by much water with separation of the free base; and salts of volatile acids behave in the same manner when their solutions are evaporated. With very weak acids, such as acetic acid, narcotine does not form salts. Consequently sodium acetate precipitates narcotine from solutions of other narcotine salts.

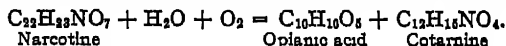


Of the opium alkaloids known, sodium acetate not only precipitates narcotine from solutions of its salts but also papaverine and narceine. It does not, however, precipitate morphine, codeine and thebaine.

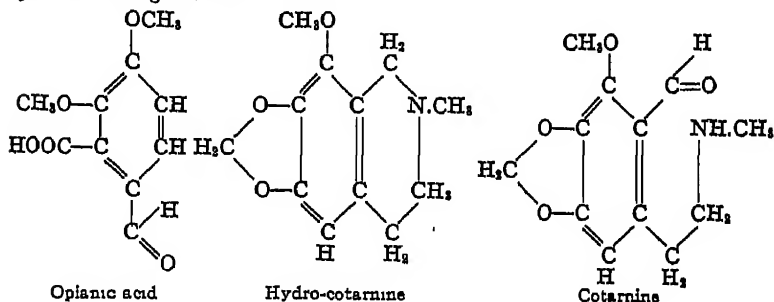
Constitution.—Narcotine is a tertiary base, for it combines with methyl iodide and other alkyl iodides forming ammonium iodides. Since by treatment with concentrated hydriodic acid, according to the method of Zeisel, three methyl-groups are split out as methyl iodide, the narcotine molecule must contain three methoxyl-groups. By hydrolytic cleavage, that is, by heating at 140° with water, with dilute sulphuric acid or barium hydroxide solution, narcotine yields nitrogen-free opianic acid and basic hydro-cotarnine which contains nitrogen.



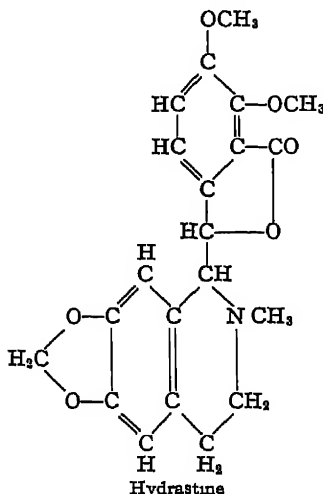
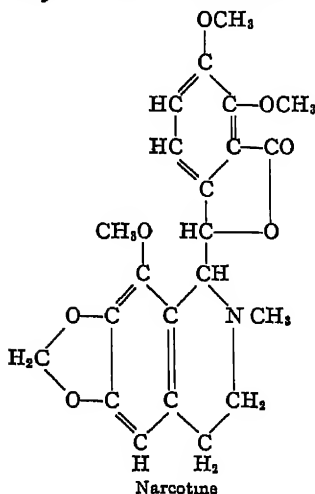
By oxidative cleavage, that is, by treatment of narcotine with such oxidizing agents as nitric acid, manganese dioxide and sulphuric acid, lead dioxide, and ferric chloride, the products opianic acid and cotarnine are obtained.



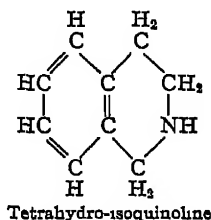
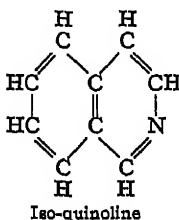
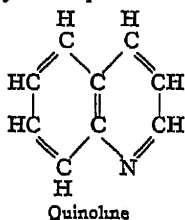
These cleavage-products clearly show that narcotine is made up of two complexes, one nitrogen-free and the other containing nitrogen. The chemical constitution of these cleavage-products has been determined and is expressed by the following formulae.



Upon the basis of their experimental results, Roser and Freund have proposed the following structural formula for narcotine which they consider as definitely determined. Narcotine is entirely analogous to hydrastine, being in fact a methoxyl-derivative of the latter.



These two alkaloids are therefore iso-quinoline derivatives, that is, of tetrahydro-isoquinoline:



Physiological Action.—Narcotine possesses only very feeble narcotic properties. Administered internally, it exerts a sedative action upon the movements of the intestines. Injected into the blood, narcotine weakens the heart and therefore lowers the blood-pressure. Together with its character as a weak sedative, narcotine like morphine exhibits a stronger stimulating action upon the central nervous system. According to Straub,¹ narcotine makes morphine more potent in its narcotic and toxic action. At the same time it produces a qualitative change, for the respiratory center is less narcotized and thus regulation of the ventilation of the body is kept more intact. The practical advantage of opium over morphine is probably due as a matter of fact to the narcotine it contains. According to Meissner,² morphine stimulates even the respiratory center

¹ W. Straub: Pharmacodynamic Action of Narcotine in Opium Biochem. Zeitschr. 41 (1912), 419.

² Meissner: Biochemisch. Zeitschr. 54 (1913), 395.

and from this point of view narcotine therefore may exert an influence antagonistic to the action of morphine. The inhibiting action of morphine upon intestinal peristalsis is not increased when combined with narcotine but is rather lessened (Trendelenburg¹). An intensification by the other opium alkaloids of the action of morphine as an anodyne is due principally to narcotine. The greatest increase is produced by the combination of a molecule each of morphine and narcotine with the dibasic meconic acid, a preparation introduced into therapy by Straub² under the name of narcophine. The morphine-content in 0.03 gram of narcophine is 0.01 gram. This preparation is soluble in water. In consequence of rather slow absorption, narcophine exhibits its action more slowly than do morphine salts but it lasts longer for that reason.

Narcotine is eliminated from the human body in part by the intestinal glands and in part by the kidneys in the urine. But according to Dragendorff it may also be detected in the stomach, liver and spleen.

Detection of Narcotine

Because of its weak basic character, narcotine can be extracted almost completely by chloroform from the aqueous tartaric acid solution, whereas ether removes only traces. This behavior differentiates narcotine from most of the other plant bases. Obviously narcotine passes from the alkaline solution into ether and chloroform. As it comes from its ether solution, this alkaloid forms a varnish-like mass having scarcely any color and hardening after a time to a mass of radiating crystals. Narcotine is precipitated from its hydrochloric or sulphuric acid solution by iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, and phospho-molybdic acid even in dilutions of 1:5000.

Special Reactions

1. **Sulphuric Acid Test.**—Narcotine dissolves with stirring in concentrated sulphuric acid, producing a greenish yellow color that gradually changes to red-yellow and finally after several days to raspberry-red.

2. **Dragendorff's Test.**—A solution of narcotine in dilute sulphuric acid (1:5), evaporated upon the water-bath in a porcelain dish, or over a very small flame, has a red-yellow color, changing with stronger heat to crimson-red. As the acid begins to evaporate, blue-violet streaks radiate from the margin and finally the entire liquid has a dirty red-violet color. The same color-changes appear, if the yellow to red-yellow solution of narcotine in concentrated sulphuric acid is heated with extreme care over a very small flame.

3. **Froehde's Test.**—This reagent dissolves narcotine with blue-green to green color. If the reagent is more concentrated, the green color immediately changes to fine cherry-red, especially upon application of gentle heat. This color is quite persistent.

4. **Mecke's Test.**—Concentrated sulphuric acid containing selenious acid dissolves narcotine with steel-blue color that becomes cherry-red upon gentle warming.

¹ P. Trendelenburg, *Archiv f. exper. Path. u. Pharmacol.* 81 (1917), 116.

² W. Straub, *Munch. med. Wochenschr.* 1912, 1542.

5. **Couerbe's Test.**—Dissolve narcotine in cold concentrated sulphuric acid and mix a trace of nitric acid with this solution after 1-2 hours. A red color will appear and gradually become more and more pronounced. Erdmann's reagent gives the same color-change. Concentrated sulphuric acid, containing 1 drop of ferric chloride solution in 10 cc, gives with narcotine upon warming a fine cherry-red color.

6. **Wangerin's¹ Test.**—Place a mixture of 0.01 gram of narcotine with 20 drops of pure concentrated sulphuric acid, and 1-2 drops of 1 per cent cane-sugar solution upon a watch-glass and heat upon the water-bath with stirring for about 1 minute. At first the solution has a greenish yellow color that passes through yellow, yellow-brown, brown and brown-violet into a very beautiful and intense, pure blue-violet. In the beginning the intensity of this color increases somewhat in the cold, then fades and becomes discolored.

Applied to apomorphine, atropine, brucine, quinine, codeine, caffeine, hydrastine, morphine, physostigmine, pilocarpine, and strychnine, this test gives solutions that are colorless or nearly so. Only the morphine solution after a while has a pale pink color. Quinine and narcotine have a light yellow color, narceme chestnut-brown; and picrotoxin salmon-color to pale pink. Colchicin, digitalin and veratrine behave toward this reagent as toward pure concentrated sulphuric acid without the addition of the small quantity of sugar.

In this test 1-2 drops of 1 per cent aqueous furfural solution may be substituted for the sugar solution. From yellow, brown, olive and other colors there finally emerges a deep, clear, dark blue. The brilliancy of this color increases somewhat upon standing. Gradually during a period of a few hours there is a change to a pure green color. For the detection of traces of narcotine (0.001 gram) use a 1 per cent sugar solution.

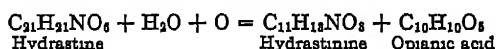
HYDRASTINE

Hydrastine, $C_{21}H_{21}NO_2$, together with berberine, $C_{20}H_{17}NO_4$ (4 per cent.) and canadine, $C_{20}H_{21}NO_4$, occurs to the extent upon the average of 1.5 per cent. in hydrastis root, the root of *Hydrastis canadensis*, almost exclusively as the free alkaloid. The fluid extract prepared from this root and used in medicine should contain according to the German Pharmacopoeia at least 2 per cent. of hydrastine.

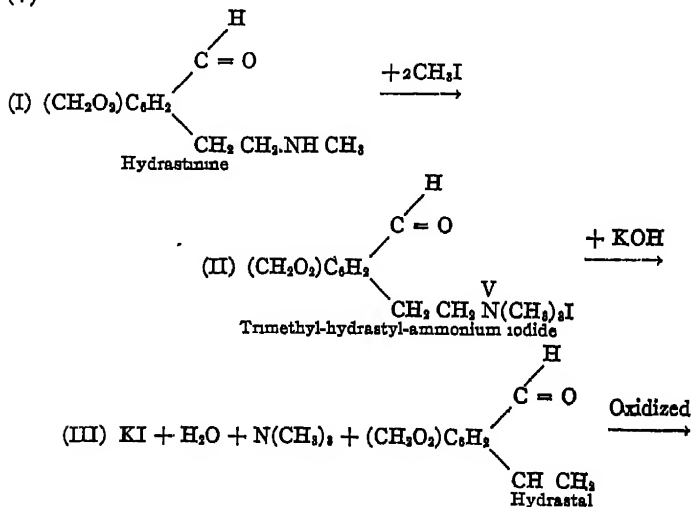
Preparation.—Extract hydrastis root with hot water containing acetic acid. Filter the extract, evaporate to a thin solution, and add dilute sulphuric acid in strong excess (about 3 volumes). Nearly all the berberine separates out in fine yellow crystals as acid sulphate, $C_{20}H_{17}NO_4 \cdot H_2SO_4$, whereas hydrastine remains in solution. Precipitate hydrastine from the filtered mother-liquor by means of ammonium hydroxide solution and purify the alkaloid by repeated crystallization from acetic ether or alcohol.

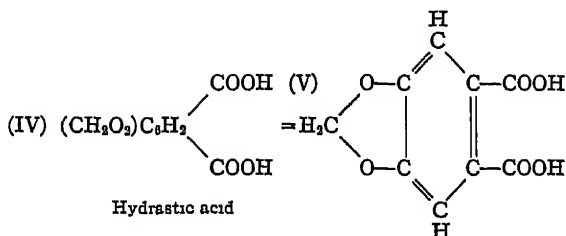
¹ A. Wangerin: Color Reactions of Narceine and Narcotine. Pharm. Ztg 48 (1903), 607.

Properties and Constitution.—Hydrastine crystallizes from alcohol in lustrous, white, rhombic prisms melting at 132° and having a bitter taste. It is nearly insoluble in water, difficultly soluble in cold alcohol but freely soluble in hot alcohol, chloroform and benzene. Ether dissolves hydrastine less easily but still sufficiently so that this solvent may be used to extract the free base from an aqueous alkaline solution. Chloroform will extract hydrastine even from the aqueous tartaric acid solution. In chloroform solution this alkaloid is laevo-rotatory, whereas in dilute hydrochloric acid it is dextro-rotatory. Hydrastine is a weak, monacid base, its salts crystallizing as a rule with difficulty. Hydrastine hydrochloride, $C_{21}H_{21}NO_6 \cdot HCl$, obtained by passing hydrochloric acid gas into a solution of hydrastine in absolute ether, forms a white crystalline powder soluble in water. Formation at 100° from its constituents of hydrastine iodo-methylate, $C_{21}H_{21}NO_6 \cdot CH_3I$, shows that hydrastine is a tertiary base. Heated according to the method Zeisel with hydriodic acid, hydrastine yields 2 molecules of methyl iodide, indicating the presence of 2 methoxyl-groups. Hydrastine undergoes oxidative cleavage with dilute nitric acid, yielding the nitrogenous base, hydrastinine, and nitrogen-free opianic acid:

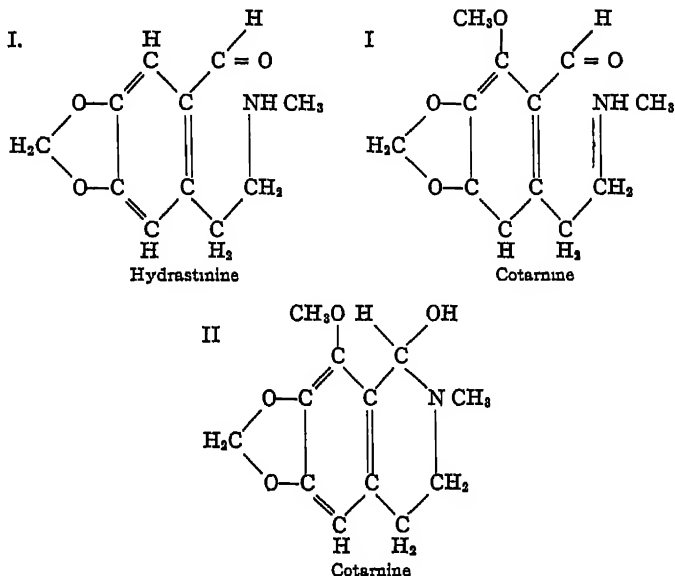


Since the constitution of opianic acid has long been known (see Narcotine, page 203), the only problem is the explanation of the nature of hydrastinine which has been determined, as well as that of many other alkaloids, by A. W. Hofmann's method of exhaustive methylation. Hydrastinine (I) is a secondary base which forms, when heated with an excess of methyl iodide, hydrastinine hydriodide and trimethyl-hydrastyl-ammonium iodide (II). Heated with alkalis, this ammonium iodide is decomposed into trimethylamine, hydriodic acid, and nitrogen-free hydrastal (III). The later upon oxidation gives hydrastic acid (IV) which was identified as the methylene ether of nor-meta-hemipinic acid (V).

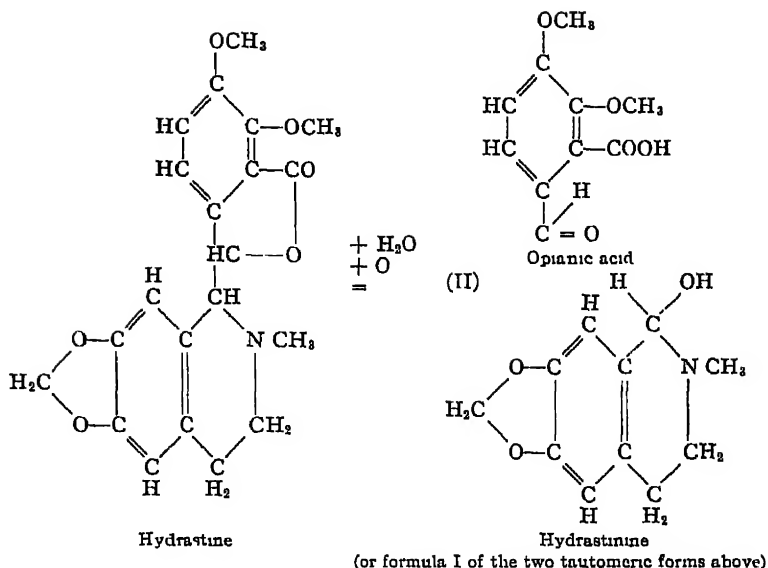




From these and other relations, it has been determined that cotarnine is a methoxy-hydrastinine. Both are able to react tautomatically



Similarly narcotine is a methoxy-hydrastine. On the basis of the structural formulae on page 203 the oxidative cleavage of hydrastine into opianic acid and hydrastinine takes place in the following manner:



Physiological Action.—According to R. Kobert, even small doses of hydrastine have a stimulating action upon the centers of the medulla oblongata in man and animals. Therefore it causes slowing of the pulse, increase in blood-pressure, and quickening of respiration. When the dose is increased, stimulation of these centers is changed to paralysis. Stimulation of the spinal cord accompanies the action upon the brain and may give rise to convulsions. Independent of this central action upon the nervous system, hydrastine causes muscular weakness of the heart. The smallest doses capable of producing an effect upon man give rise to increased blood-pressure. Hydrastinine on the other hand is not toxic at all for the muscles of the heart but rather increases the activity of the heart. Very small doses raise in man the tonus of the blood-vessels, especially when they are abnormally inactive. Large doses paralyze the blood-vessels and the heart-vagus after previous stimulation and cause death by paralyzing respiration. Hydrastinine has a stimulating action upon the isolated surviving uterus of many animals, strengthening its normal movements and in larger doses occasioning tetanus. Cotarnine, the nitrogenous cleavage-product of narcotine, acts upon the uterus like hydrastinine but has no action whatever upon the blood-vessels. Preparations of hydrastis and cotarnine are used to control abnormal flow of blood at menstruation.

Detection of Hydrastine

1. **Concentrated Sulphuric Acid.**—This acid dissolves hydrastine without color but the solution becomes violet when gently warmed.
2. **Froehde's Test.**—This reagent dissolves hydrastine with green color that gradually changes to brown.

3. **Mandelin's Test.**—Sulphuric acid containing vanadic acid dissolves hydrastine with rose-red color, immediately changing to orange-red and gradually fading

4. **Fluorescence Test.**—Hydrastine dissolved in dilute sulphuric acid gives a colorless solution that does not fluoresce (distinction from quinine) Add very dilute potassium permanganate solution drop by drop, shaking well after each addition. Hydrastine is formed and the solution exhibits beautiful blue fluorescence

Ether extract of the aqueous alkaline solution, obtained by the Stas-Otto method, usually leaves hydrastine in a crystalline condition

QUININE

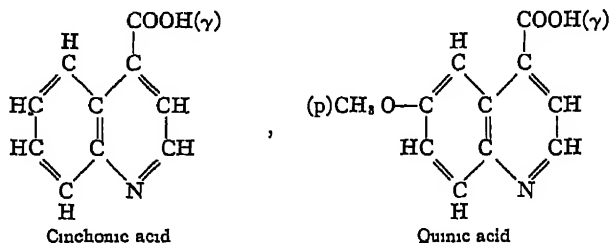
Quinine, $C_{20}H_{24}N_2O_2$, occurs together with cinchonine and other alkaloids as salts of quinic and quinotannic acid, especially in the barks of various species of *Cinchona*. These various barks show a very wide range in the amount of quinine they contain. The South American barks rarely contain more than 5 per cent and frequently only 2–3 per cent of quinine, whereas the bark of *Calsaya Ledgeriana* from Java has repeatedly been found to contain as high as 13 per cent.

Properties.—Addition of ammonia to the solution of a quinine salt first produces a white, cheesy, amorphous precipitate of anhydrous quinine. After a short time, however, it takes up 3 molecules of water and changes to crystalline quinine hydrate, $C_{20}H_{24}N_2O_2 \cdot 3H_2O$. This compound may be obtained in long, silky needles by crystallization from dilute alcohol, or if a solution of quinine in strong ammonia is saturated in sealed tube at 100° and allowed to cool slowly. Anhydrous quinine separates from solution of quinine hydrate in strong alcohol, ether and benzene. The former may be obtained in the form of silky needles by dissolving quinine hydrate in hot dilute alcohol and allowing the solution to stand for a long time at 30° . Quinine hydrate melts at 57° . Heated at higher temperature, it solidifies again and then melts at 171° , the melting-point of anhydrous quinine. Anhydrous quinine dissolves at 15° in 1960 parts of water and the hydrate in 1670 parts and in about 900 parts of boiling water. The solubility of this base in water is increased by addition of ammonia but decreased by sodium or potassium hydroxide solution. Alcohol, ether, benzene and carbon disulphide are good solvents of quinine. Quinine solutions are laevo-rotatory, the degree of rotation varying considerably according to concentration and solvent. Alcoholic or aqueous solutions of quinine salts, prepared by means of sulphuric, nitric, phosphoric, formic, acetic, tartaric or citric acid, exhibit fine blue fluorescence. But fluorescence does not appear with hydrochloric, hydrobromic and hydriodic acid. In fact addition of one of these acids to solutions of the other salts even inhibits fluorescence. Chlorides, bromides and iodides

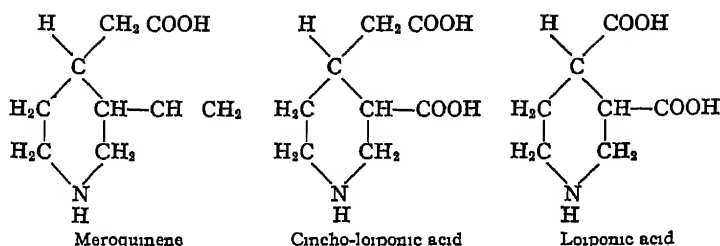
have the same effect. Concentrated sulphuric acid dissolves quinine giving a colorless or at most a pale yellowish color

Constitution.—Quinine is a diacid, ditertiary base, combining with one and two equivalents of acid and forming salts that usually crystallize well. The salts with one equivalent of acid are the more stable. Quinine hydrochloride, $C_{20}H_{24}N_2O_2 \cdot HCl \cdot 2H_2O$, used in medicine, crystallizes in long, delicate tufts of needles. Quinine sulphate, the officinal quininum sulphuricum, $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 8H_2O$, forms white silky needles. Aqueous and alcoholic solutions of these two salts have a neutral reaction, whereas solutions of the so-called acid quinine hydrochloride, $C_{20}H_{24}N_2O_2 \cdot 2HCl$, and acid quinine sulphate, or quinine bisulphate, $C_{20}H_{24}N_2O_2 \cdot H_2SO_4 \cdot 7H_2O$, have a strong acid reaction.

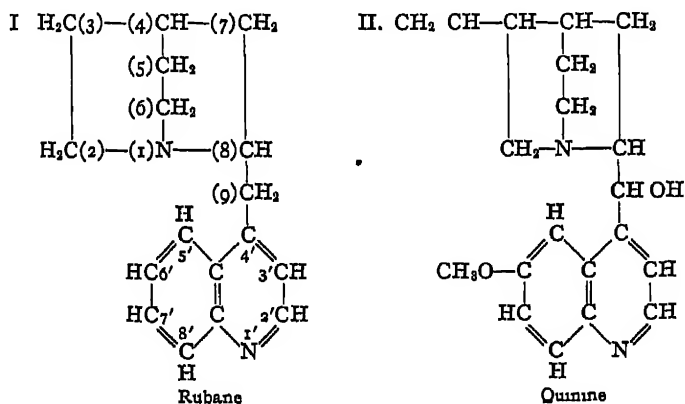
In accordance with its ditertiary character, quinine can combine with 2 molecules of an alkyl iodide, for example, it forms with methyl iodide quinine di-iodo-methylate, $C_{20}H_{24}N_2O_2 \cdot 2CH_3I$. The quinine molecule also contains a hydroxyl-group, for it gives a monacetyl and a monobenzoyl derivative. The presence of one methoxyl-group may also be detected by Zeisel's method. Therefore the empirical formula of quinine may be resolved into $C_{19}H_{20}(OH)(OCH_3)N_2$. The empirical formula of quinine, $C_{20}H_{24}N_2O_2$, differs from that of cinchonine, $C_{19}H_{22}N_2O$, by (CH_2O) . Consequently quinine must be regarded as a methoxy-cinchonine, for the methoxyl-group cannot be detected in the cinchonine molecule. Upon oxidation with chromic acid, cinchonine gives cinchonic acid, identical with quinoline- γ -carboxylic acid, and quinine under the same conditions gives quinic acid, or p-methoxy-cinchonic acid.



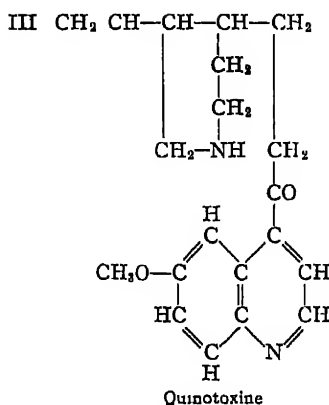
Both alkaloids upon oxidation also give nitrogenous compounds, meroquinene, cincho-loiponic acid and loiponic acid. Consequently there is no doubt that cinchonine and quinine contain two nitrogenous nuclei, one of which is a quinoline complex. The second nucleus is connected with the latter in the γ -position, as shown by formation of cinchonic and quinic acid. Meroquinene, cincho-loiponic acid and loiponic acid, derived by oxidation with chromic acid from the so-called "second half" of the cinchonine and quinine molecule, form a continuous series of oxidation products, since meroquinene can be oxidized to cincho-loiponic acid and the latter to loiponic acid. The following formulae offer the most satisfactory explanation of the relationship existing between these three compounds.



Rabe,¹ whose researches in the field of the cinchona alkaloids have met with success, has recently introduced the name "rubane" for the mother-substance from which quinine and related alkaloids are derived. Cinchona alkaloids have thus far been found only in plants belonging to the family of the Rubiaceae. This mother-substance, rubane, is a disubstituted methane and consequently is designated as (quinuclidyl-8)-(quinolyl-4'). The position of substituents in the quinoline nucleus is designated in the usual manner by α, β, γ , ortho, etc., or better by the primed numerals 1', 2', 3', etc., and in the other rings by the number 1-9, beginning with the nitrogen atom of the quinuclidine nucleus and ending with the C-atom attached to the quinoline nucleus in the γ -position, as shown in formula I, that of the mother-substance, rubane. Quinine, formula II, in accordance with this nomenclature would be 6'-methoxy-3-vinyl-rubanol-(9) and quinotoxine, formula III, easily derived from it as 6'-methoxy-3-vinyl-rubatoxanan-(9). This representative of the cinchona-toxines was earlier called according to Pasteur "Quinicine" which was later changed by v. Miller and Rhode to quinotoxine.



¹ P. Rabe: Contribution to the Knowledge of Cinchona Alkaloids, XXIII: Nomenclature and Phenomena of Isomerism. Ber d Deutsch chem. Ges. 55 (1922), 922.



Physiological Action.—Quinine is a pronounced protoplasmic poison and in sufficiently large doses paralyzes and destroys not only different unicellular organisms, such as plasmodia, but also ciliated cells, white and red blood-corpuscles, muscle cells, nerve cells, plant cells and enzymes. The partition process of the cell nucleus is deranged by quinine (O Hertwig). Bacteria and molds are paralyzed only by relatively large doses, and under the same conditions fermentations are unfavorably influenced. Schmiedeberg assumes that quinine in rather small doses increases the vitality and heightens the capacity of the particular cells in their functions. Quinine diminishes frequency of heart-beat even in presence of atropine. The blood-vessels of isolated surviving organs are greatly dilated by quinine (Kobert). For this reason and also because of paralysis of vaso-motor centers, blood-pressure drops. As a result of dilatation of cutaneous blood-vessels there is increased loss of heat. It is not impossible that oxidation ferments, which control processes of combustion in the tissues, are also concerned in lowering of efficiency. The so-called quinine-drunk is probably due to lowering of the efficiency of the cortex of the brain. It manifests itself as heavy stupor of the head. In the case of individual animal species death ensues during paralysis of the respiratory center accompanied by great weakness of heart action. In man convulsions may appear in quinine coma (Kobert).

The symptoms of quinine poisoning are: abdominal pains, emesis, diarrhoea, ringing in the ears, difficulty in hearing, deafness, permanent blindness, disturbances of speech, paralysis of the limbs, chills, cold sweats, and severe collapse lasting several days. There is also swelling of the face and tongue, and skin eruptions (quinine exanthema) are somewhat frequent even after rather small doses. Haemoglobinuria, methaemoglobinuria (black water fever), icterus, vomiting of blood, bloody diarrhoea, albuminuria lasting for weeks, fever, and cyanosis have been observed. A fatal case of poisoning by quinine, observed by Strzyzowski and Petroff, is described in the literature. A woman 22 years old with suicidal intent took 15 grams of quinine sulphate and drank the juice of two lemons which facilitated solution of the quinine salt. After about 2 hours she felt very weak, had ringing in the ears, dizziness, emesis and looked pale.

After 4 hours she was wholly unconscious and had convulsions. The pulse was small but regular, respiration normal. After 5 hours severe convulsions, action of the heart weak and irregular, pupils unreactive, extremities cool, repeated emesis. After 6 hours continued convulsions during which death ensued. In cases followed by recovery, derangement of sight and hearing on both sides persisted for weeks or months or for life.

Quinine hydrochloride is absorbed from all body cavities, from subcutaneous tissues and from the gastro-intestinal tract. After use of quinine, metabolism is at first increased for a short time and then considerably diminished. As a result elimination of nitrogen drops.

Elimination in Man.—Quinine, administered internally in daily doses of 0.5 gram of quinine hydrochloride, is eliminated for the most part unchanged in the urine. Schmitz¹ was unable to find in the urine transformation products of quinine, such as dihydroxy-quinine described by Kerner. Within 48 hours 20-30 per cent of quinine, taken by the mouth in the form of powdered hydrochloride, was eliminated. After continued administration of quinine, the quantity eliminated daily in the urine varied between 19 and 35 per cent, averaging about 26 per cent. Elimination of quinine in urine appears to be less when used subcutaneously than when taken by the mouth, averaging daily only 16 per cent. That portion of quinine not appearing in the urine is apparently destroyed in the organism. After prolonged use of quinine, the power of the human body to destroy this alkaloid does not seem to increase, according to results of experiments made by Schmitz. Details with regard to detection and quantitative estimation of quinine in urine are given in Chapter V (see page 552).

Traces of quinine may also be found in saliva, perspiration and milk. In animals, after subcutaneous injection of considerable doses, a large part of the quinine is eliminated in the gastro-intestinal tract and appears in the faeces scarcely altered in form.

In the few cases, ending acutely in death, the lethal dose amounted to 8-15 grams of quinine hydrochloride; but in one case recovery followed after 15 grams of this quinine salt and in another after even 20 grams.

Detection of Quinine

Ether, benzene or chloroform extracts quinine from aqueous alkaline solution. Upon evaporation, ether deposits the alkaloid as a resinous, amorphous varnish in which its presence may be recognized by the following tests:

1. **Fluorescence Test.**—Dilute sulphuric acid dissolves the residue from ether solution and produces a fine blue fluorescence if quinine is present. Even in a dilution of 1:100,000 this fluorescence is distinctly visible.

¹ R. Schmitz: Elimination of Quinine in Human Urine. Arch. f. exper. Path. u. Pharmak. 56 (1907), 301.

2. Thalleioquin Test.—Dissolve the residue from the ether solution in a little dilute acetic acid and add 5–10 drops of saturated chlorine water. The colorless solution has a faint blue fluorescence and will at once give a fine green color upon addition of ammonia in excess, if quinine is present. Larger quantities of quinine give a green precipitate, thalleioquin. It is always obtained as an amorphous substance of variable composition. Thalleioquin is soluble in alcohol and chloroform but insoluble in ether. E. Pollacci heats quinine (about 0.01 gram) gradually to boiling with 2–3 cc. of water, a little lead dioxide, and 2 drops of dilute sulphuric acid. The clear solution after settling is decanted or filtered and carefully covered with 5–6 drops of ammonia. A green zone will appear at the contact-surface of the two liquids.

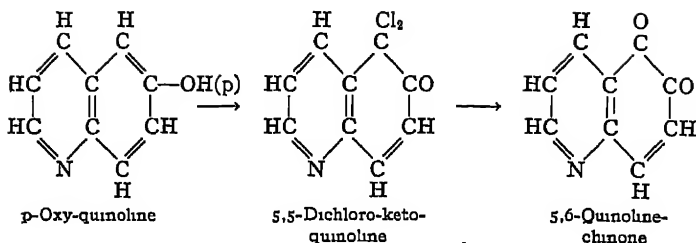
According to Abensour the thalleioquin test may be made more easily and with greater certainty, if carried on in a neutral or only faintly acid alcoholic solution and if the dyestuff is dissolved in a little chloroform. Add to 10 cc. of the solution to be tested saturated bromine water drop by drop until the fluorescence has disappeared. Then dilute the mixture with the same volume of alcohol and add 1–2 drops of ammonia. The green dyestuff may also be taken up in chloroform. When the material is a quinine salt, dissolve in a little alcohol, add an equal volume of water, and proceed as described above.

Interferences with the Thalleioquin Test.—Antipyrine interferes with this test.

Mixtures of 1 per cent solutions of antipyrine and quinine give finally a fine red instead of a green color. This interference does not cease until these two substances are in the proportion of 0.25 part of antipyrine to 5 parts of quinine. Caffeine also interferes with the thalleioquin test, when the proportion is 2 parts of quinine to 3 parts of caffeine. Other compounds, such as urea, prevent appearance of this color, whereas atropine, codeine, cocaine, morphine, pilocarpine, strychnine, as well as carbolic acid and chloral hydrate, have no effect upon the thalleioquin test.

Fühner¹ has shown that the thalleioquin reaction is connected with the p-oxy-quinoline complex. Chlorine passed into a solution of pure p-oxy-quinoline hydrochloride cooled with ice produces a white crystalline precipitate. This substance crystallizes from petroleum ether in colorless prisms or plates melting at 58°. Structurally it is 5,5-dichloro-6-keto-quinoline. Solutions of this dichloro-keto-quinoline and of its hydrochloride are colored pure green or blue by ammonia. Fühner thinks 5,6-quinoline-quinone is probably formed and gives the green color with ammonia.

¹H. Fühner, Thalleioquin Reaction of Quinine and the Kynurenic Acid Reaction of Jaffé. *Ber. d. Deutsch. chem. Ges.* 38 (1905), 2713.



3. Herapathite Test.—Mix 30 drops of acetic acid, 20 drops of absolute alcohol, and 1 drop of dilute sulphuric acid (20 per cent. H_2SO_4) Add 20 drops of this mixture to 0.01 gram of quinine and heat to boiling. Finally add 1 drop of alcoholic solution of iodine (1:10), or 2 drops of 0.1 n-iodine solution. At once, or sometimes not until the solution has stood for some time, green leaflets having a metallic lustre separate. This product is so-called “herapathite,” an iodine compound of quinine having the constant composition, $4\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2 \cdot 3\text{H}_2\text{SO}_4 \cdot 2\text{HI} \cdot 4\text{I} \cdot 3\text{H}_2\text{O}$. It may be recrystallized from boiling alcohol. Herapathite crystals are pale olive-green by transmitted light but by reflected light have a fine, cantharidin-green, metallic lustre. They have a strong polarizing action upon light.

Caustic alkalis, ammonia, sulphurous acid and hydrogen sulphide decompose herapathite. A Christensen recommends keeping on hand the following reagent for the herapathite test: 1 part of iodine; 1 part of 50 per cent. hydriodic acid, 0.8 part of sulphuric acid, and 50 parts of 70 per cent. alcohol. Add a few drops of this reagent to the alcoholic solution to be tested for quinine.

4. Abensour's¹ Erythroquinine Test.—Add 1 drop each of half-saturated bromine water, potassium ferrocyanide solution (1:10), and ammonia (10 per cent. NH_3) to 10 cc. of a faintly acid, best with acetic acid, solution of quinine. This mixture, if shaken, gradually turns red. This test is given especially well, if the mixture is at once shaken with chloroform. This solvent takes up the color and has the appearance of a chloroform solution of iodine. This is an exceedingly delicate test for quinine. Chloroform takes on a brilliant red-violet color even with 1 cc. of a 1:100,000 quinine solution = 0.01 mg. of alkaloid.

Of the alkaloidal reagents, potassium bismuthous iodide is especially recommended as a precipitant of quinine. With quinine sulphate solutions, this reagent produces precipitates having an intense

¹ J. Abensour. Detection of Quinine. Journ. de Chim. et Pharm. (6) 26 (1907), 25.

yellow-red color. Shaken with sodium hydroxide solution, this precipitate is decomposed and unaltered quinine may be obtained by extraction with ether and evaporation of the ether solution. Thoms¹ has made use of this reaction in the quantitative separation of quinine from mixtures.

CAFFEINE

Since caffeine is a weak base, ether extracts only a little of this alkaloid from tartaric acid solution. The greater part is extracted by ether, or better chloroform, from aqueous alkaline solution. Ether usually deposits caffeine in white, shining needles arranged in clusters. Since caffeine is rather difficultly soluble in ether, the aqueous alkaline solution should be repeatedly extracted with large quantities of this solvent. For tests characteristic of this alkaloid see page 148.

ANTIPYRINE

In the Stas-Otto process most of the antipyrine is extracted by ether from aqueous alkaline solution. It is usually purer from alkaline than from acid solution and frequently appears in crystalline leaflets. Antipyrine differs from most alkaloids in having only a faintly bitter taste and in being freely soluble in water. To identify antipyrine, dissolve the ether residue in a little water and divide the solution into two equal parts. Test one portion with ferric chloride solution and the other with fuming nitric acid. Further details with regard to tests for antipyrine will be found on page 142.

PYRAMIDONE

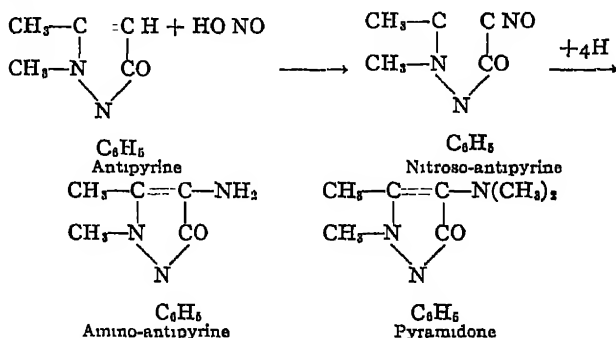
Pyramidone, or 4-dimethyl-amino-antipyrine, $C_{13}H_{17}N_3O$, has been extensively used of late as an antipyretic and anodyne. Its effects are like those of antipyrine but its action is 3-4 times stronger, so that a correspondingly smaller dose, that is, 0.25-0.3 gram, may be taken.

Properties.—Pyramidone is a white, crystalline powder having a faintly bitter taste and melting at 108° . It dissolves in about 30 parts of cold and more easily in hot water. It is freely soluble in alcohol (1:2), in chloroform (1:1), and somewhat less in ether (1:9). The aqueous solution of pyramidone has a faint alkaline

¹D Jonescu and H Thoms. Precipitation and Quantitative Estimation of Alkaloids by Potassium Bismuthous Iodide. Ber. d. Deutsch. pharm. Ges. 16 (1906), 130.

reaction. Pyrimidone is a stronger base than antipyrine and consequently only traces pass from tartaric acid solution into ether. It differs from antipyrine in being a strong reducing agent. Pyrimidone, for example, will reduce gold chloride even in the cold, whereas antipyrine and tolpyrine require heat.

Preparation.—Antipyrine dissolved in concentrated acetic acid is converted by potassium nitrite into nitroso-antipyrine appearing as green crystals. This compound in alcoholic solution is reduced by zinc dust and acetic acid to amino-antipyrine. The latter, treated with methyl iodide and potassium hydroxide in methyl alcohol solution, is converted into dimethyl-amino-antipyrine = pyrimidone.



Detection of Pyrimidone

Pyrimidone can be easily and completely extracted by ether or chloroform from aqueous alkaline solution. Upon evaporation of solvent it usually appears in fine needles. Pyrimidone solutions give precipitates with most of the general alkaloidal reagents. Because of its strong reducing properties, it reacts especially easily with oxidation agents, such as ferric chloride, iodine and silver nitrate. Pyrimidone may be identified by the following tests:

1. **Nitrous Acid Test.**—An aqueous pyrimidone solution, acidified with dilute hydrochloric or sulphuric acid, exhibits a temporary blue-violet color with a little potassium nitrite solution. This color disappears gradually; rapidly upon further addition of potassium nitrite. This test is useful in detecting antipyrine in pyrimidone, for the former gives quite a stable green color.

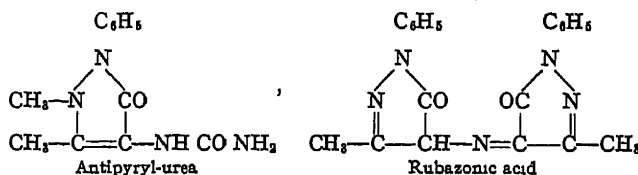
2. **Ferric Chloride Test.**—With ferric chloride solution aqueous pyrimidone solutions give the same blue-violet color produced by iodine solution. This color at once passes into red-violet and soon disappears.

3. **Silver Nitrate Test.**—Aqueous pyrimidone solutions, treated with silver nitrate solution, first have an intense blue color due to

formation of colloidal silver. After a while the liquid becomes dark and metallic silver separates

Pyrimidone finds therapeutic use in the form of salts and molecular compounds, such as the salicylate, melting at 68–76°, and trigemine, a compound of pyrimidone with butyl-chloralhydrate, melting at 85°.

Behavior in the Human Organism and Elimination.—Following internal administration of pyrimidone, the urine is usually colored bright purplish red. It contains a conjugated glycuronic acid, rubazonic acid and antipyril-urea. Even after large doses, unchanged pyrimidone cannot be found in the urine. After standing for some time, pyrimidone-urine frequently deposits rubazonic acid as a sediment, consisting of red needles soluble in ether or chloroform but especially in acetic ether. The solvent has a ruby-red color. If pyrimidone-urine is acidified, rubazonic acid may be extracted with acetic ether. Formation in the human organism of rubazonic acid and antipyril-urea from pyrimidone is due to demethylation, as may be seen by comparing the formulae below with that of pyrimidone given above. Therefore the same processes go on in the case of pyrimidone as in that of the purine bases, caffeine, theobromine and theophylline, from which in the course of animal metabolism methyl-groups also attached to nitrogen undergo cleavage, at least in part.



Detection of antipyril-urea and rubazonic acid in pyrimidone-urine, according to the method of Jaffé, is taken up in Chapter V of this book (see page 566)

Fatal poisonings from pyrimidone are thus far unknown. In some instances this pyrazolone derivative has caused gastric disturbance and been responsible for such marked secretion of sweat that further administration of pyrimidone had to be abandoned. (Meyer-Gottlieb)

C. Examination of Ether and Chloroform Extracts of Ammoniacal Solution

(α) **Ether Extract.**—Apomorphine and traces of morphine.¹

(β) **Chloroform Extract.**—Morphine and narceine. Antipyrine, colchicin and caffeine² may also be in this extract.

¹ Ether dissolves traces of freshly precipitated, amorphous morphine

² Antipyrine, colchicin and caffeine, though freely soluble in chloroform, dissolve with difficulty in ether. If these substances are not completely extracted by ether from the aqueous alkaline solution, they will be present in the chloroform extract together with morphine and narceine. Colchicin in particular will be obtained at this point in a fairly pure condition.

The preliminary test for morphine and apomorphine is given on page 223.

The aqueous alkaline solution (see page 106), separated from ether, must be tested further for substances mentioned under α and β . Apomorphine may be recognized by the green color of the original aqueous tartaric acid solution. Excess of sodium hydroxide solution causes oxidation, especially if the solution is exposed for any time to air, and gradually changes the color to purple-red. Moreover, ether extracts, both of the acid and alkaline solutions, are red or violet-red when apomorphine is present. If the aqueous and ether solutions, obtained by the Stas-Otto method, do not have the characteristics mentioned, examination for apomorphine is unnecessary, that is, extraction with ether may be omitted. In that case proceed at once with the examination for morphine and narceine.

To extract apomorphine, morphine and narceine with the proper solvent, the aqueous solution separated from ether, which is alkaline from sodium hydroxide, should be rendered alkaline with ammonium hydroxide solution. First acidify the solution with dilute hydrochloric acid (test with blue litmus paper) and then add ammonium hydroxide solution until alkaline.

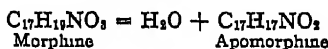
(α) If there is indication of apomorphine, extract the aqueous ammoniacal solution immediately with ether and, after removing the ether extract, repeatedly with hot chloroform.

(β) If there is no indication of apomorphine, extract the ammoniacal solution direct several times with hot chloroform (see below)

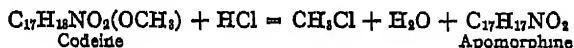
(α) Ether Extract

APOMORPHINE

Apomorphine is derived from morphine by loss of 1 molecule of water under the influence of hydrochloric, sulphuric or oxalic acid, zinc chloride, as well as the alkalis:

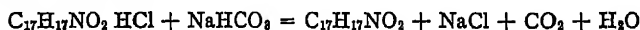


Codeine also gives apomorphine when heated at 140° with concentrated hydrochloric acid:



Preparation.—Heat morphine (1 part) in sealed tube for about 3 hours at 140 – 150° with 25 per cent hydrochloric acid (10 parts). Cool the contents of the tube, add sodium bicarbonate in slight excess, excluding air as much as possible,

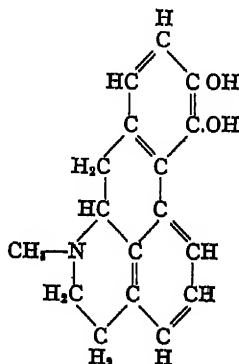
and immediately extract with ether or chloroform, Morphine, that has not undergone change, remains undissolved. Addition of a little concentrated hydrochloric acid to the ether or chloroform solution causes crystallization upon the walls of the container of apomorphine hydrochloride (Apomorphinum hydrochloricum) used in medicine. The free base apomorphine may be precipitated from this salt, after it has been purified by crystallization from a little hot water, by sodium bicarbonate



Properties.—Apomorphine freshly precipitated is white and amorphous. In contact with air in moist condition it turns green. It is somewhat soluble in water and dissolves easily in alcohol, ether and chloroform. Aqueous and alcoholic solutions of apomorphine, which are colorless when freshly prepared, turn green in the air as a result of oxidation. Aqueous and alcoholic solutions of apomorphine thus changed by oxidation are emerald-green. Ether and benzene solutions are purple-violet, those in chloroform blue-violet. As a phenol, apomorphine is readily soluble in sodium or potassium hydroxide solution. A solution of the alkaloid in potassium hydroxide solution absorbs oxygen from the air and turns brown. Apomorphine differs from morphine, especially in solubility in ether, benzene and cold chloroform. Morphine is almost insoluble in these solvents.

Constitution.—Apomorphine is a monacid, tertiary base and at the same time a di-acid phenol. As such it is easily soluble in solutions of caustic alkalies. The presence of two phenolic hydroxyl-groups is shown by the fact that it gives in alkaline solution with benzoyl chloride colorless, crystalline needles of dibenzoyl-apomorphine, $\text{C}_{17}\text{H}_{15}\text{N}(\text{O} \cdot \text{CO} \cdot \text{C}_6\text{H}_5)_2$, and with diazo-methane dimethyl-apomorphine, $\text{C}_{17}\text{H}_{15}\text{N}(\text{O} \cdot \text{CH}_3)_2$.

Since dimethyl-apomorphine, after previous exhaustive methylation, gives an intermediate product that is oxidized by potassium permanganate to dimethoxy-phenanthrene carboxylic acid, apomorphine like morphine is to be regarded as a derivative of phenanthrene, $\text{C}_{14}\text{H}_{10}$ (Pschorr). Upon the basis of his experimental results, Pschorr¹ has proposed for apomorphine the accompanying structural formula.



¹ R. Pschorr, Problem of the Constitution of Apomorphine. Ber. d. Deutsch. chem. Ges. 39 (1906), 3124; and Ibid. 40 (1907), 1984.

Physiological Action.—Apomorphine frequently finds therapeutic use as an expectorant and emetic but not as a narcotic. In man after use of medicinal doses, emesis is the only evidence of central stimulation. In a case described in the literature, a man had taken 0.2 gram of apomorphine hydrochloride. He fainted repeatedly, had painful breathing and a feeling of suffocation until finally abundant emesis occurred and the pain disappeared. Intense dizziness, falling down, deathly pallor, cold sweats and rattling in the throat may appear (Loeb). According to Bergell and Pschorr, the emetic action of apomorphine is due to presence of the two hydroxyl-groups. Substances formed by esterifying or etherifying these groups are without emetic properties. It is a notable fact that different classes of animals do not behave in the same manner toward apomorphine. Dogs have emesis after 0.1 mg. of apomorphine and do not die even after 2 grams but are only narcotized and paralyzed for a rather long time. Apomorphine does not produce emesis at all in swine, cattle and horses but has a highly stimulating action. Many horses like many persons are specifically susceptible and die even after relatively small doses of apomorphine.

Detection of Apomorphine

Ether will not extract apomorphine from a solution containing tartaric acid but will dissolve its colored oxidation products. This solvent behaves similarly toward solutions of this alkaloid containing caustic alkalies. Ether or chloroform will extract apomorphine only from a solution alkaline with ammonium hydroxide or sodium bicarbonate. Ether solutions of apomorphine usually deposit a greenish, amorphous residue. This alkaloid has a strong reducing action. It will reduce iodic acid with liberation of iodine which may be detected by means of chloroform. It will produce a purple color with gold chloride due to metallic gold. Most of the tests that follow are the result of oxidation of apomorphine by which green, violet or blue oxidation products are formed. This is true of the tests with iodine, ferric chloride, potassium dichromate, potassium ferricyanide, or atmospheric oxygen.

1. **Sulphuric and Nitric Acids.**—Concentrated sulphuric acid dissolves apomorphine without color. Addition of a drop of concentrated nitric acid to this solution produces a fugitive violet color, changing at once to blood-red and finally to yellow-red. Concentrated nitric acid alone with apomorphine gives a violet-red solution that immediately becomes red-brown and brown-red.

2. **Pellagri's Test.**—Dissolve apomorphine in dilute hydrochloric acid and add sodium bicarbonate to faint alkaline reaction. Then add drop by drop 1-3 drops of dilute alcoholic iodine solution, shaking well after each addition. The color of the solution will be

blue-green or emerald-green. Extract with ether (2-3 cc.) and this solvent will have a fine violet-red color, whereas the aqueous solution will remain green.

3. **Froehde's Test.**—This reagent dissolves pure apomorphine with green color, or violet if the alkaloid has been altered by air.

4. **Wangerin's¹ Test.**—Prepare a fresh solution of apomorphine hydrochloride (about 1 per cent.). Add 4 drops of potassium dichromate solution (0.3 per cent.) to 1 cc. of this solution and shake for about 1 minute. The solution will be deep dark green. Then add a few cc. of acetic ether and shake again. This solvent will become violet. Now add about 5 drops of stannous chloride solution² (1 per cent.) and shake well. The color of the acetic ether layer will change to green and, upon further addition of a few drops of potassium dichromate solution, the acetic ether will again become violet. If 10 cc. of chloroform are substituted for acetic ether in this test, the oxidation products of apomorphine will impart the same violet color to the chloroform. But if stannous chloride solution is carefully added, the color will change to pure indigo-blue and persist when shaken again with potassium dichromate solution.

5. **Schmidt's³ Tests.**—The following especially typical tests are recommended for detection of apomorphine hydrochloride:

(a) A drop of dilute ferric chloride solution (1:10) will turn 10 cc. of an aqueous apomorphine hydrochloride solution blue even in dilution of 1:10,000.

(b) Shake 10 cc. of the same apomorphine hydrochloride solution with 1 cc. of chloroform. Then render alkaline with sodium hydroxide solution and at once shake well with air. The aqueous solution acquires a fugitive violet color and the chloroform is blue.

6. **Feinberg's⁴ Test.**—Dissolve apomorphine hydrochloride in not too little water, add 3 drops of 1 per cent. potassium ferricyanide solution, and shake well with 1 cc. of benzene. The color of the benzene layer will be amethyst. Addition of a few drops of dilute

¹ A. Wangerin: *Helch's Pilocarpine Test and Apomorphine Reactions*. Pharm. Ztg. 47 (1902), 599 and 739 to 740.

² Dissolve 1 gram of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 50 cc. of pure 25 per cent. hydrochloric acid and bring the volume to 100 cc. when cold with pure dilute hydrochloric acid.

³ E. Schmidt: *Apomorphine Hydrochloride*. Apotheker-Zeitung 23 (1908), 657.

⁴ M. Feinberg: *Contributions to the Knowledge of Apomorphine*. Zeitschr. f. physiol. Chem. 84 (1912), 363.

sodium carbonate solution produces with agitation first a violet-red that changes after long standing to a fine violet color. This is a very delicate test, capable of detecting 0.00003 gram of apomorphine in 1 cc. of solution, and morphine and other opium alkaloids cause no interference.

(3) Chloroform Extract

Preliminary Morphine Test.—As a preliminary test for morphine, acidify a small portion of the aqueous alkaline solution (see page 107), separated from ether with dilute sulphuric acid, add a few drops of iodic acid solution, and extract with a little chloroform. If the latter is violet from iodine, morphine may be present. But a final decision regarding presence of morphine should not be made from a positive test, since many other organic substances besides this alkaloid will reduce iodic acid.¹ This is a delicate preliminary test for morphine and that is its only value. If no reaction takes place, probably morphine is not present, if positive, morphine may be present. Apomorphine also reduces iodic acid.

To detect morphine and narceine with certainty, render the aqueous solution alkaline with ammonia, or sodium bicarbonate, and extract at once in a capacious flask as already directed (see page 107) with considerable hot chloroform.² Separate the two liquids as usual in a separating funnel. Several extractions of the aqueous solution with fresh portions of hot chloroform, or hot chloroform-alcohol mixture, are necessary because of the slight solubility of morphine even in boiling chloroform. Should the chloroform and aqueous solution form a troublesome emulsion that will not separate, add a few drops of alcohol, set the flask upon a warm but not boiling water-bath, and carefully rotate the flask from time to time. This procedure usually causes immediate separation of the two liquids. Place the combined chloroform extracts in a dry flask, add a few crystals of dry sodium chloride, or anhydrous sodium sulphate, to remove adherent water, pour the chloroform when clear through a dry filter, and evaporate in not too large a

¹ In testing animal matter containing absolutely no morphine, the author has repeatedly obtained extracts that strongly reduced iodic acid.

² C. Kippenberger (Contributions to the Analytical Chemistry of Alkaloids. *Zeitschr. f. analyt. Chem.* 39 (1900), 201, 290) extracts morphine with chloroform, containing 10 per cent. by volume of absolute alcohol. In several instances the author has found this chloroform-alcohol mixture very useful and prefers it at the present time to pure chloroform.

glass dish upon a warm water-bath. The chloroform may also be filtered direct into the dish as fast as it evaporates. If the residue is bitter and can be scraped together with a platinum spatula or a pocket-knife, test for morphine and narceine.¹ In testing for morphine, use Froehde's, Husemann's and Pellagri's test, as well as that given by formalin-sulphuric acid. Presence of morphine is not established unless all these morphine tests give positive results! If the quantity of residue from chloroform permits, test for morphine with ferric chloride solution. This test is very characteristic of morphine but requires more than traces for a satisfactory result. Finally make Straub's biological test for morphine.

Purification of Impure Morphine

When the chloroform residue is too impure, especially if red or brown, it should be purified. Dissolve it in hot amyl alcohol and shake the solution thoroughly with several portions of hot water containing a few drops of dilute sulphuric acid. Acid dissolves morphine, whereas amyl alcohol retains most of the coloring matter. Add ammonium hydroxide solution, or sodium bicarbonate, to the acid solution until alkaline and extract several times with hot chloroform. Morphine obtained by evaporation of chloroform should be nearly pure.

Extraction of Free Morphine with Ether-Alcohol Mixture

To extract free morphine, Jörgensen² recommends ether containing alcohol, because it removes less extractive matter, the source of impurity of extracts from cadaveric material, than do the usual morphine solvents, such as amyl alcohol, chloroform or acetic ether. Slow evaporation of the clear ether extract gives as a rule entirely colorless, prismatic crystals of morphine that respond to the tests for this alkaloid in a manner admitting of no doubt. To prevent alcohol as much as possible from passing from ether into water, only 1-1.5 per cent of alcohol is added to the ether, since too much alcohol in water will retard or entirely prevent passage of morphine into ether. Since ether containing alcohol is far inferior to the usual solvents of morphine, the number of extractions of the ammoniacal solution should be increased. At least ten such extractions are required. If the aqueous solution is quite impure, first remove morphine by extraction with hot amyl alcohol and then take the alkaloid from the latter by extraction with water containing hydrochloric acid. Add ammonia in excess to this acid solution and extract morphine with ether containing 1-1.5 per cent of alcohol.

¹ Antipyrine, colchicin and caffeine may also be in this residue.

² G. Jörgensen. Detection of Morphine in Animal Organs. *Zeitschr. f. analyt. Chem.* 49 (1910), 484.

According to the original instructions of the Stas-Otto method, free morphine is extracted not with hot chloroform but with amyl alcohol. Aside from the fact that it is unpleasant to work with this alcohol on account of its disagreeable odor, amyl alcohol is decidedly toxic. As a result of inhaling even small quantities of its vapor, sensitive persons may have nausea and severe pains in the head. In the experience of the author, morphine obtained from extracts with commercial amyl alcohol is not as pure and free from color as from chloroform extracts. For this reason years ago the author recommended warm chloroform for the extraction of morphine. In following the Stas-Otto method, Nagelvoort¹ recommends isobutyl instead of commercial amyl alcohol, since it is as good a solvent of morphine as the latter and is without its unpleasant odor.

MORPHINE

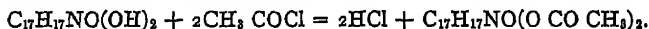
Opium contains several alkaloids combined almost exclusively with sulphuric and meconic acid. Of these alkaloids morphine is always present in largest quantity, that is, to the extent of 10 per cent. and more. In addition to its occurrence in the juice of the unripe capsules of the colored flowers of different varieties of *Papaver somniferum*, morphine is also found in smaller quantity together with other opium bases in all parts of these plants, such as leaves, stems and seeds, but only preceding maturity. As the ripening process advances, the juice in the plant diminishes and also the quantity of morphine, so that ripe poppy-heads contain at most only traces of the alkaloid.

Properties.—Morphine, $C_{17}H_{19}NO_3 \cdot H_2O$, crystallizes from dilute alcohol with 1 molecule of water of crystallization. It forms colorless, transparent, lustrous prisms that are only slightly soluble in water (1.5000 at 15° and 1.500 at 100°). These solutions are very bitter and have an alkaline reaction. Crystalline morphine is insoluble in ether and benzene. The amorphous alkaloid is soluble in amyl alcohol, isobutyl alcohol, hot chloroform and acetic ether. Ammonia, caustic alkalies, alkaline carbonates and bicarbonates precipitate the free base from solutions of morphine salts but, since morphine is a phenol, excess of caustic alkali holds it in solution.

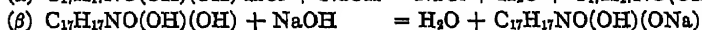
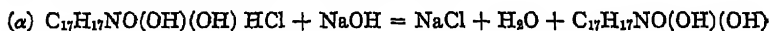
Constitution.—Morphine as a monacid base combines with one equivalent of acid, forming as a rule salts that crystallize well. Their solutions have a neutral reaction and a very bitter taste. Morphine hydrochloride (*Morphinum hydrochloricum*), $C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$, the most important salt of this alkaloid, is extensively used in medicine. The tertiary character of morphine appears from the fact that it combines with methyl iodide in sealed tube at 100°, forming morphine-methyl iodide, $C_{17}H_{19}NO_3 \cdot CH_3I$, crystallizing in colorless lustrous needles that yield upon treatment with moist silver oxide the slightly stable, crystalline ammonium base, morphine-methyl hydroxide, $C_{17}H_{19}NO_3 \cdot CH_3 \cdot OH$.

¹ J. B. Nagelvoort: Detection of Alkaloids in Toxicological-chemical Examinations. *Nederl. Tijdschr. Pharm.* 10 (1898), 616.

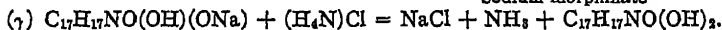
$5\text{H}_2\text{O}$. Fusion of morphine with caustic potash, or heating with soda lime, gives methyl amine, showing that a methyl-group is attached to nitrogen, that is, that this alkaloid contains the N CH_3 group. The three oxygen atoms possess different functions. Two form hydroxyl-groups, for with acetyl chloride morphine forms diacetyl-morphine, $\text{C}_{17}\text{H}_{17}\text{NO}(\text{O CO CH}_3)_2$, the hydrochloride of which is used in medicine under the name "Heroin".



Similarly, benzoyl chloride gives dibenzoyl-morphine, $\text{C}_{17}\text{H}_{17}\text{NO}(\text{O CO C}_6\text{H}_5)_2$, crystallizing in needles. One hydroxyl-group must be phenolic in character, for morphine exhibits the properties of a monacid phenol. It is soluble in solutions of caustic alkalis but not in ammonia and for that reason is precipitated from these alkaline solutions by ammonium salts.

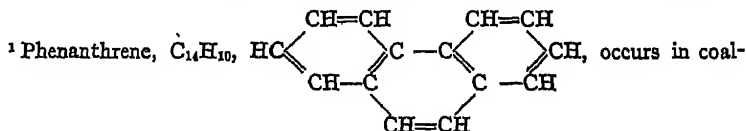


Sodium morphinate



The phenolic character of morphine is shown by the fact that its neutral salts give a characteristic coloration with ferric chloride and that diazonium salts couple with morphine in alkaline solution forming azo-dyestuffs. The second oxygen atom of morphine is alcoholic in character; and the third is in ether-like union with two carbon atoms, a so-called bridge-oxygen atom.

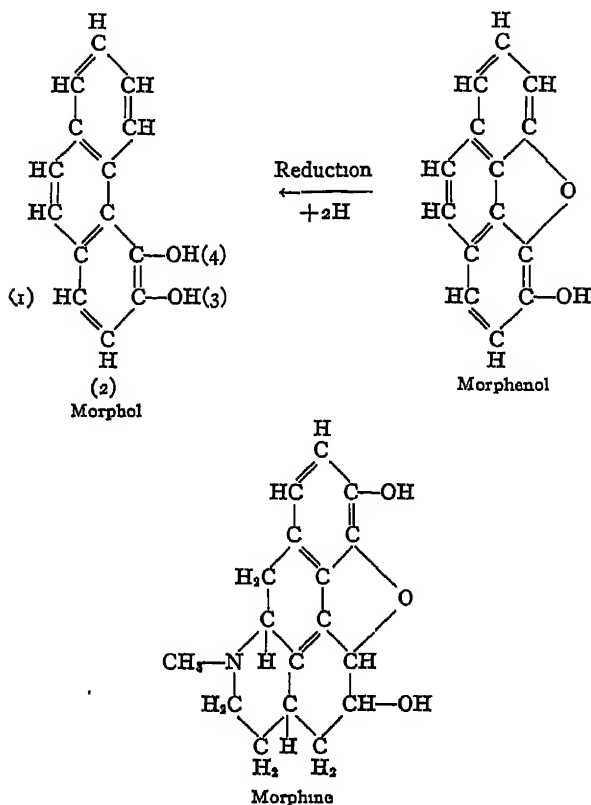
Of the 17 carbon atoms in morphine, 14 belong without question to a phenanthrene nucleus, for when morphine is heated with ten times the quantity of zinc dust, it gives off considerable ammonia and methyl amine, and a thick, brown liquid distils over consisting principally of phenanthrene.¹ More than that, well-defined phenanthrene derivatives have been obtained from derivatives of morphine by various reactions. If morphine-methyl iodide (see above) is boiled for several hours with acetic anhydride, the product together with other substances is diacetyl-dioxyphenanthrene, $\text{C}_{14}\text{H}_8(\text{O CO CH}_3)_2$, which gives morphol, $\text{C}_{14}\text{H}_8(\text{OH})_2$, upon treatment with alcoholic ammonia. This compound has been prepared synthetically by Pschorr² and is identical with 3, 4-dioxyphenanthrene. A second derivative of phenanthrene is obtained from methyl-codeine methyl iodide, or from the corresponding ammonium base, which undergoes cleavage during dry distillation into trimethyl-amine, ethylene and methyl-morphenol. The latter heated with hydriodic acid is converted into morphenol,



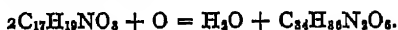
tar together with its isomer anthracene. It forms colorless crystals melting at 99° and boiling at 340° . It is readily soluble in ether or benzene and with difficulty in alcohol. Phenanthrene solutions exhibit bluish fluorescence.

² R. Pschorr. Constitution of Apomorphine. A Contribution to the Problem of the Constitution of Morphine. *Ber. d. Deutsch. chem. Ges.* 40 (1907), 1984.

$C_{14}H_7O(OH)$, which easily gives phenanthrene upon distillation with zinc dust and morphol upon reduction with sodium in absolute alcohol solution. Morphenol contains two hydrogen atoms less than morphol. The constitution of morphol has been definitely established by synthesis (Pschorr) and that of morphenol is probably as represented below. At the same time the formula of morphine proposed in 1907 by R. Pschorr is included



Morphine is easily oxidized. This may be brought about in alkaline solution by atmospheric oxygen. Potassium permanganate, potassium ferricyanide, ammoniacal copper solution, and ammoniacal silver nitrate solution may also be used. As a result non-toxic oxy-dimorphine, also called pseudo-morphine which is soluble in caustic alkali, is formed:



The strong reducing action of morphine is due to the ease with which it undergoes oxidation.

Physiological Action.—Morphine is easily absorbed by the mucous membrane of the stomach, rectum, respiratory tract and open wounds. The action of

morphine is more rapid and stronger following hypodermic injection than when administered by way of the stomach. The entirely different reaction toward morphine of various animals is remarkable. Cold-blooded vertebrates are very slightly sensitive to morphine. To produce effects in a frog, more of the alkaloid must be injected than is required by man. Birds (pigeons and hens) can also stand surprisingly large doses of morphine. According to Guinard, the average toxic dose of morphine per kilogram of body-weight is 7 mg. for a horse, 15 mg. for a beef-creature, 40 mg. for a cat, 65 mg. for a dog, 200 mg. for a hog, 320 mg. for a rabbit, and 400 mg. for a goat. Considerable qualitative differences in the action of morphine have also been observed, since the brain of one group is primarily stimulated and of another group primarily paralyzed. At a second stage in the case of the second group increase in reflex-irritability of the spinal cord appears. In response to morphine, man is in the second group. Since his brain is exceedingly sensitive to poisons causing paralysis, the most pronounced action observed is that of a sedative and hypnotic (Kobert). In Europeans the stage of brain-stimulation seldom is noted, whereas it is said to occur more frequently in Malays and other inferior races. The stage of stimulation of the spinal cord in human adults is rarely observed, for long before that they die from paralysis of respiration. Morphine exerts a marked influence upon regulation of the temperature of the human body. If the environment is cold, the temperature drops below normal; if warm, fever appears. Under the influence of morphine, consumption of oxygen and escape of carbon dioxide are considerably diminished. During severe intoxication from morphine, respiration is slow and superficial.

Symptoms of morphine poisoning are soon in evidence, rarely later than 1-2 hours, and manifest themselves by: great somnolence, insensibility, deepest cyanosis, coma becoming deeper and deeper, pulse imperceptible and very slow, respiration rattling or entirely imperceptible, and body-temperature far below normal. Additional symptoms observed are first very brief dilatation of the pupils, then extreme contraction, and before death recurrence of dilatation. Death usually ensues in 6-8 hours from pulmonary oedema and cessation of respiration. In morphine poisoning terminating favorably, coma gradually gives place to quiet sleep often lasting 24-36 hours. The patient after awaking usually has headache and frequently emesis. In many cases constipation and digestive disturbances are very persistent. For an adult the smallest lethal dose of morphine is 0.2 gram and the average 0.4 gram. On the other hand, opium-eaters and morphinists frequently tolerate several grams of morphine daily, since the organisms of such individuals can dispose of quite large quantities of morphine. There is habituation to the poison!

Elimination.—According to Marquis,¹ morphine disappears very rapidly from the blood, since it is held by certain organs, such as the brain. A portion of the morphine absorbed is conjugated with glycuronic or sulphuric acid (Baumann), another portion is oxidized, and the balance of the alkaloid is eliminated unchanged. After medicinal doses only little morphine, or none at all, appears in the urine. The urine of morphinists, however, is different. In such urines

¹ E. Marquis: *Continuance of Morphine in the Animal Organism*. Investigations of the Dorpat Institute edited by Kobert 14 (1896).

the author has repeatedly found quite considerable quantities of this alkaloid by the colorimetric method. In man and dogs a not immaterial part of the morphine taken is again eliminated by the glands of the gastro-intestinal tract and also when the alkaloid is injected subcutaneously.

According to Cloetta,¹ morphine in acute poisonings is carried on in the blood-plasma but completely disappears, 20 minutes at least after injection. Decomposition of morphine in the circulation is said not to occur. Lipoids of the brain abstract morphine from the plasma and there it is rather firmly held. One consequence of this is serious disturbance of the function of brain cells, but on the other hand the morphine molecule also undergoes decomposition. That part of the morphine not held by the brain is decomposed elsewhere in the body or eliminated. The ability of the organism to destroy morphine in acute poisoning differs with individuals. Oxidative processes play an important part in the decomposition of morphine. According to Cloetta, ferment action is excluded. In habituation the power of lipoids of the brain to combine with morphine increases, but at the same time increase in decomposition of the alkaloid takes place.

Contrary to the statements of Cloetta, Rübsamen² was unable to detect decomposition of morphine by the brain substance of normal animals outside the body. During acute morphine poisoning, vomitus, the stomach and contents, intestines, faeces, and especially the urine should be examined. Usually morphine can also be easily detected in the saliva. According to Marquis, the parts of the cadaver best suited for detection of morphine are liver and kidneys.

Stability of Morphine in Cadaveric Putrefaction.—According to Autenrieth,³ morphine is only slightly decomposed, even during putrefaction lasting for a long time. In the stomach and contents of an adult, who took 25 grams of tincture of opium and died 4 hours later, he found 0.028 gram of nearly pure morphine. The urine was also found to contain morphine. After 18 months of continuous putrefaction, he found in what was left of the cadaveric material 0.02 gram of morphine, referred to the original quantity of material. Doepmann⁴ after 11 months detected unchanged morphine in putrid meat to which it had been added. He extracted the meat with water containing acetic acid, evaporated the filtrate to small volume, precipitated with alcohol, and distilled the alcohol from the filtered liquid. The solution of the residue in water was filtered and precipitated with basic lead acetate. The filtrate from this precipitate was freed from lead by hydrogen sulphide. After removal of lead sulphide, the filtrate containing acetic acid was evaporated to about 100 cc. made alkaline with ammonia, and repeatedly extracted with hot chloroform. In the residue from chloroform Doepmann detected morphine positively by Husemann's and

¹ M. Cloetta. Behavior of Morphine in the Organism and Causes of Habituation to this Alkaloid. Arch. f. exper. Path. u. Pharm. 50 (1903), 453.

² W. Rübsamen: Experimental Studies of Habituation to Morphine. Arch. f. exper. Path. u. Pharm. 59 (1908), 227.

³ W. Autenrieth: Behavior of Morphine and Strychnine in Cadaveric Putrefaction. Ber. d. Deutsch. pharmaz. Ges. 11 (1901), 494.

⁴ F. Doepmann: Experiments on Stability of Morphine during Putrefaction. Chem.-Ztg. 39 (1915), 69.

Pellagri's tests, by its behavior with Froehde's and Marquis' reagents, and also by Straub's biological test

With regard to the stability of morphine during cadaveric putrefaction, Grutterink and van Rijn¹ have shown that they could detect this alkaloid with certainty after it had been in a cadaver for 25 years

In an examination of the products of bacterial metabolism, human organs, consisting of liver, spleen, kidneys, heart, pancreas, intestines, etc., were subjected both to aerobic and anaerobic putrefaction, but no product was obtained giving tests that could be mistaken for those of morphine. Moreover these bacterial degradation-products do not interfere in any way with the detection of morphine. This alkaloid was added to mixtures undergoing putrefaction and could be easily detected by tests admitting of no doubt.²

Detection of Morphine

Evaporation of the chloroform, or alcohol-chloroform extract, leaves morphine as an amorphous, varnish-like residue, that only rarely becomes crystalline and has a very bitter taste. It should be carefully scraped together with a platinum or nickel spatula, or the clean blade of a pocket-knife, to get an idea of the approximate amount of substance available for tests. If the quantity is small, only specific tests for morphine should be made.

General alkaloidal reagents especially sensitive to solutions of morphine salts are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, gold chloride and phosphomolybdic acid. Platonic chloride produces an orange-yellow, granular precipitate only in concentrated solutions and then not until some time has elapsed.

Special Tests for Morphine³

1. **Nitric Acid Test.**—Concentrated nitric acid dissolves morphine with blood-red color changing at once to yellow. Addition of stannous chloride, or ammonium sulphide, fails to produce a violet color in this yellow solution (distinction from brucine).

2. **Husemann's Test.**—Concentrated sulphuric acid dissolves morphine without color. Heat this solution at once in a watch-glass for about 30 minutes upon a water-bath, or for a short time over a very small flame, until copious white fumes appear. A reddish

¹ A. Grutterink and W. van Rijn: Morphine in Parts of Cadavers. *Pharm. Weekblad* 52 (1915), 423.

² J. Rosenbloom and S. R. Mills: Non-conformity of Ptomaines with Certain Morphine Reactions. *Journ. of Bio. Chem.* 16 (1914), 327.

³ Carry out these tests with free morphine base obtained by precipitation by ammonia from the aqueous solution of hydrochloride.

or more of a brownish color is produced. Cool this solution and add a drop of concentrated nitric acid. A fugitive red-violet color appears, almost instantly changing to blood-red and yellow-red and finally fading out entirely. Instead of warming the solution of morphine in concentrated sulphuric acid, allow it to stand in a desiccator at ordinary temperature for about 24 hours and then add a drop of concentrated nitric acid. A small crystal of potassium nitrate or chlorate may be substituted for nitric acid.

Frequently impure morphine is obtained from the chloroform extract of a solution prepared from cadaveric material. Such a residue gives a more or less highly colored solution with sulphuric acid. Heat usually intensifies this color. But even under these conditions it is possible to detect the red color caused by nitric acid or potassium nitrate.

3. Pellagri's Test.—Proceed with this test as described under codeine (see page 200). Avoid excess of alcoholic iodine solution, otherwise the latter may mask the green color.

Husemann's and Pellagri's tests depend upon conversion of morphine by concentrated sulphuric acid into apomorphine and detection of this base.

4. Froehde's Test.—This reagent dissolves morphine with a fine violet color, passing through blue into dirty green and finally into faint red. These colors vanish upon addition of water.

5. Marquis' Test.—Concentrated sulphuric acid containing formaldehyde dissolves morphine with a purple-red color, gradually passing into violet and finally into pure blue. This blue solution, kept in a test-tube and only slightly exposed to air, retains its color for some time.

Codeine and apomorphine give the same violet color. Narcotine also gives violet solutions but they become olive-green and finally yellow. Oxy-dimorphine gives a green color.

6. Ferric Chloride Test.—A few drops of neutral ferric chloride solution produce a blue color in a neutral solution of a morphine salt. This color gradually fades. It is due to the oxidizing action of ferric chloride upon morphine.

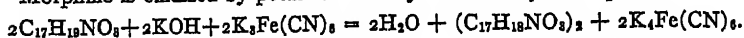
In making this test dissolve the chloroform residue in very dilute hydrochloric acid. Evaporate this solution to dryness upon the water-bath. Dissolve the residue in pure water and add a drop of ferric chloride solution.

7. Iodic Acid Test.—Shake a dilute sulphuric acid solution of morphine with a few drops of aqueous solution of pure iodic acid.

(HIO_3), or potassium iodate (KIO_3) free from potassium iodide, and a little chloroform. The latter will have a violet color.

8. Brouardel-Boutmy Test.—An aqueous solution of a morphine salt is colored deep blue by a few drops of a very dilute mixture of potassium ferricyanide and ferric chloride solution. Considerable morphine will produce a blue precipitate of Prussian blue.

Morphine is oxidized by potassium ferricyanide to oxy-dimorphine:



Potassium ferrocyanide formed at the same time reacts with ferric chloride producing Prussian blue

The basis of the tests with iodic acid and potassium ferricyanide (Tests 7 and 8) is the reducing action of morphine. Therefore other reducing substances, such as hydrastine, also give these two tests. There are also many ptomaines that exert a strong reducing action and consequently give the Brouardel-Boutmy test

9. Nitrous Acid Test.—Add a small particle of sodium nitrite to a very dilute morphine salt solution, then a dilute acid and render alkaline with concentrated potassium hydroxide solution before all the gas has escaped. According to the concentration of the morphine solution, the mixture will be pale rose, orange-red to deep ruby-red. Acids discharge and alkalies restore the color.

In this test nitroso-morphine, $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_4 \cdot \text{H}_2\text{O}$, is probably formed. Wieland¹ has obtained this compound in pure condition by passing nitrous acid (prepared from arsenic trioxide and concentrated nitric acid) into an aqueous solution of morphine hydrochloride cooled to about -3° . The sodium compound from 90 per cent alcohol forms garnet-red needles. Free nitroso-morphine, obtained from this alkali salt with acetic acid, crystallizes in dark orange-red needles melting at 225°

10. Donath's Test.—Triturate at least 1 mg. of morphine in a small porcelain dish with 8 drops of concentrated sulphuric acid. Add a small particle of potassium arsenate, triturate again, and warm over a small flame until fumes of sulphuric acid appear. A blue-violet color, passing into dark brown-red after further warming, will appear. Careful dilution with water develops a reddish color that becomes green upon addition of more water. If the latter is shaken in a test-tube with a little chloroform, this solvent will acquire a fine violet color.

¹ H. Wieland and P. Kappelmeier Researches upon Morphine I. *Annalen d. Chemie* 382 (1911), 306.

11. Lloyd's Test.—Triturate morphine (5–8 parts) with hydrastine (1 part) and a few drops of concentrated sulphuric acid with a glass rod for 5–10 minutes. In the center the mixture is red-violet and blue-violet in the thinner marginal region. This color is similar to that produced by potassium dichromate in a sulphuric acid solution of strychnine. Apomorphine also gives Lloyd's test. Aconitine, atropine, berberine, brucine, and other alkaloids also give colors with hydrastine and sulphuric acid. Wangerin¹ considers these reactions characteristic only when 5–10 mg. of morphine and 2–10 mg. of hydrastine are present.

12. Schneider-Weppen Test.—Add to a colorless concentrated sulphuric acid solution of morphine a small particle of cane-sugar, or add morphine mixed with about 4 times the quantity of finely powdered sugar to concentrated sulphuric acid. The mixture has a red color that becomes more intense upon addition of a drop of bromine water.

13. Flückiger's Test.—Sprinkle upon a solution of morphine in concentrated sulphuric acid a little subnitrate of bismuth. A black-brown color will appear.

14. Azo-dyestuff Test.—Morphine is a phenol and will couple with diazonium salts forming azo-dyestuffs.

Dissolve morphine in dilute acetic acid. Cool well and add a cold diazonium salt solution prepared from aniline and containing sufficient sodium acetate to discharge mineral acid. Then drop in ice-cold sodium carbonate solution until the reaction is alkaline. A cinnabar-red body appears, soon changing into stable phenyl-azomorphine, $C_{22}H_{23}N_3O_3$, crystallizing from hot alcohol in orange-yellow needles (H. Wieland).

By diazotized sulphanilic acid, morphine may be determined quantitatively by the colorimetric method. Details of this method are given in Chapter V of this book (see page 563).

15. Biological Test of Straub and Hermann.²—If an aqueous solution of morphine hydrochloride is injected under the skin of the back of a white mouse, after a few minutes the back takes on lordotic curvature. Slight spastic paresis = incomplete paralysis appears in the hind legs, so that they are held more strongly than usual in an extended position and it appears difficult for the animal to move. The tail begins to rise in an S-shaped curve until it finally lies over the

¹ A. Wangerin: Contribution to Lloyd's Reaction for Morphine. *Pharm. Ztg.* 48 (1903), 57.

² O. Hermann: A Biological Method of Detecting Morphine. *Biochem. Zeitschr.* 39 (1912), 216.

back of the animal so that the tip is over the ears. The entire behavior of the mouse gives the impression of increased restlessness and reflex-irritability, so that it jumps up terrified at faint noises, especially from acoustic stimulation of high sounds. The position of the tail is most striking and regular and is increased by running about. It is advisable to place the animals under a bell-jar upon a wooden plate and alarm them by striking the jar with a glass rod. Use white mice weighing 16-20 grams. After injection of 5 mg. of morphine the reaction lasts about 20 hours, falling rather uniformly, according to the dose, to 45 minutes after 0.005 mg. The duration of the maximum of the reaction amounts to 4.5 hours for 5 mg., falling to 30-45 minutes for 0.01 mg. of morphine. From a forensic point of view this reaction by itself is not sufficiently convincing. Yet it may be used as a preliminary test, or to confirm what chemical tests have shown to be probable. Its practical application is due chiefly to the fact that crude morphine obtained by the Stas-Otto method may be used direct for the biological test, thus avoiding purification of the alkaloid which is usually attended with considerable loss of material.

In Chapter V of this book detection of morphine in animal organs according to the procedures of Cloetta and Marquis is explained in full.

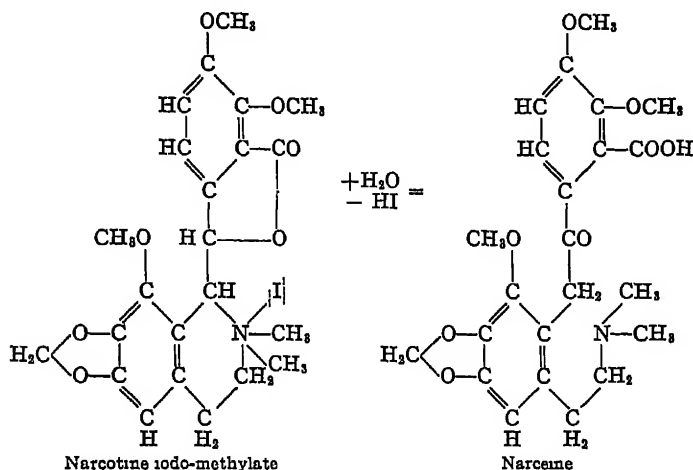
NARCEINE

Narceine is found in opium to the extent of 0.1-0.4 per cent. In chemical nature it is closely related to narcotine from which it may be prepared (see below).

Properties and Constitution.—Narceine, $C_{23}H_{27}NO_3 \cdot 3H_2O$, crystallizes from water or alcohol in long, white prisms frequently in clusters, melting when air-dried at 165° . This alkaloid has a faintly bitter taste. It is slightly soluble in cold but freely soluble in hot water. When a hot saturated aqueous solution of narceine is cooled, it solidifies to a crystalline mass. Narceine is insoluble in ether, benzene or petroleum ether and only slightly soluble in cold alcohol, amyl alcohol and chloroform, though freely soluble in these solvents at boiling temperature. Solutions of narceine react neutral to litmus and are optically inactive. Narceine is more abundantly soluble in aqueous ammonia, or caustic alkalies, than in pure water.

Narceine is a tertiary base in which two methyl-groups are attached to nitrogen, for it combines with alkyl iodides to form ammonium iodides. With methyl iodide it gives narceine iodo-methylate, $C_{23}H_{27}NO_3 \cdot CH_3I$. By Zeisel's method it may be shown that the molecule also contains three methoxyl-groups. Since narceine is soluble in caustic alkalies and ammonia and also forms esters with alcohols, it must contain a carboxyl-group. This alkaloid must also contain a carbonyl-group (CO), since it forms a hydrazone with phenyl-hydrazine. The narceine molecule contains neither an alcoholic nor a phenolic hydroxyl-group, since it forms no acetyl-derivative when heated with acetic anhydride. Upon the basis of these facts the narceine formula may be resolved into $C_{18}H_{11}NO(CH_3)_2(OCH_3)_3(CO)(COOH)$. From a chemical point of view narceine is closely related to narcotine. By heating narcotine iodo-methylate with sodium hydroxide solution, Roser converted it into a base called pseudo-

narceine Freund has recently shown that Roser's pseudo-narceine is identical with the opium alkaloid narceine and explains the conversion of narcotine into narceine by assuming that the iodo-methylate loses a molecule of hydriodic acid and takes up a molecule of water



All reactions and transformations of narceine may be easily explained upon the basis of this structural formula

Physiological Action.—The action of narceine is similar to that of morphine but milder. It is probably only slowly absorbed, for it can be found for quite a long time in the stomach and intestines. Elimination takes place principally in the urine and bile. Dragendorff has also found it in the blood and in organs rich in blood. In doses of 0.01–0.02 gram narceine is used as a sedative and antispasmodic, and as a hypnotic in doses of 0.03–0.1 gram. This alkaloid is used in the form of the free base and as the hydrochloride and meconate. The combination sodium narceinate-sodium salicylate ($\text{C}_{23}\text{H}_{23}\text{NO}_2\text{Na} + 3\text{C}_6\text{H}_4(\text{OH})\text{COONa}$) is used in medicine under the name "Antispasmine." Secondary effects, observed after doses of 0.03–0.1 gram of narceine, are: dryness in the mouth, emesis, slowing of heart-action, dysuria, itching of the skin, and increased secretion of sweat (Lewin)

Detection of Narceine

Narceine possesses at the same time the characteristics of a weak base and of a weak acid. Consequently it acts like a base with the stronger acids and like an acid with strong bases, forming salts that are, however, in aqueous solution extensively hydrolyzed. This amphoteric character of narceine explains why it cannot be extracted by ether, benzene or petroleum ether from a solution that is either acid or alkaline from sodium hydroxide. Extraction takes place better, if, as in the case of morphine, the solution is rendered alkaline with ammonia or sodium bicarbonate and shaken with hot chloroform or amyl alcohol. According to Kippenberger, chloroform containing 10 per cent. of alcohol even in the cold

completely extracts narceine from a solution alkaline from sodium bicarbonate. The general alkaloidal reagents that are more sensitive to narceine are: iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide and phospho-molybdic acid.

Special Reactions of Narceine

1. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves narceine with a yellow to yellow-brown color, changing in the cold within a few hours, and immediately upon warming, to blood-red

2. **Dilute Sulphuric Acid Test.**—If narceine is warmed in a small porcelain dish upon the water-bath with dilute sulphuric acid, at a certain concentration the acid takes on a fine violet color, changing to cherry-red upon longer heating. If a trace of nitric acid is added to this cherry-red solution when cold, blue-violet streaks will appear

3. **Froehde's Test.**—This reagent dissolves narceine with a brown-green color, gradually passing into green and finally into red. Gentle heat favors this color-change. If somewhat larger quantities of narceine are warmed until the color is red and the solution is allowed to cool, a fine corn-flower blue will extend out from the margin

4. **Erdmann's Test.**—This reagent, as well as concentrated nitric acid, dissolves narceine with yellow color

5. **Vogel's Test.**—Pour a few drops of chlorine water upon narceine and add, while stirring, a few drops of ammonia. A deep red color at once appears, persisting in presence of excess of ammonia and upon warming.

6. **Iodine Test.**—Aqueous iodine solution (iodine water), or iodine vapor, colors solid narceine blue. Morphine strongly influences the delicacy of this reaction.

7. **Potassium Zinc Iodide Test.**—With 0.5 mg. of narceine, even in dilution of 1:1000, this reagent produces a precipitate of white, filiform crystals that turn blue after a time. This blue color appears immediately, if a trace of iodine solution is added to the reagent.

8. **Resorcinol-Sulphuric Acid Test.**—Rub together upon a watch-glass 0.01–0.02 gram of resorcinol with 10 drops of concentrated sulphuric acid, add a trace of narceine (0.002–0.005 gram), and while stirring warm the intensely yellow solution upon a boiling water-bath. A fine crimson-red to cherry-red color will appear. As the solution cools, this color, beginning at the margin, gradually passes through blood-red and after several hours into orange-yellow.

9. **Tannin-Sulphuric Acid Test.**—Heat upon a water-bath while stirring 0.002–0.01 gram of narceine with 0.01–0.02 gram of tannin and 10 drops of concentrated sulphuric acid. The solution at first yellow-brown immediately becomes pure green. If heat is applied for some time, the green color changes to blue-green and finally through a more or less blue tone to a dirty green.

Narcotine and hydrastine give a similar green color-reaction with tannin-sulphuric acid. In chemical structure these two alkaloids are related to narceine. Veratrine gives a red color, whereas most of the other alkaloids give more or less brown colors that are not characteristic. (Wangerin.¹)

¹ A. Wangerin: Color Reactions of Narceine. *Pharmaz.* 47 (1902), 916.

Behavior of More Important Alkaloids toward Concentrated Nitric Acid, Concentrated Sulphuric Acid, and Reagents¹ of Froehde, Marquis, Mandelin, Mecke, Rosenthaler and Schaer

The more important alkaloids give with the above reagents colors that are more or less characteristic

Pure Concentrated Nitric Acid (Sp. Gr. 1.40 = 61 per cent.) dissolves:

Berberine. red-brown,	Narcotine: lemon-yellow,
Brucine: blood-red, immediately yellow,	Papaverine: yellow, then orange,
Codeine yellow to orange,	Physostigmine yellow,
Colchicin violet, then dirty greenish brown,	Strychnine yellow,
	Thebaine yellow
Morphine red-yellow,	Veratrine: faintly yellowish.
Narceine fugitive yellow,	

The following are soluble without color: aconitine, atropine, quinine, caffeine, cocaine, coniine, nicotine, theobromine and cytisine.

Pure Concentrated Sulphuric Acid dissolves:

Berberine: olive-green, then yellow,	Papaverine: violet-blue,
Digitalin orange-yellow and red,	Physostigmine. yellow, soon green.
Emetine. pale brownish,	Picrotoxin orange-yellow,
Colchicin. yellow	Solanine reddish yellow,
Curarine. red,	Thebaine blood-red, later yellow-red,
Narcotine: pale yellow, gradually yellow-red,	Veratrine: yellow with green fluorescence, then orange and cherry-red.
Narceine: yellow, soon brown-yellow,	

If not warmed, the following are soluble without color: atropine, brucine, quinine, hydrastine, codeine, caffeine, cocaine, morphine, nicotine, pilocarpine, strychnine, theobromine and cytisine.

Froehde's Reagent, or Molybdic-sulphuric Acid² dissolves:

Berberine: brown-green,	Narceine yellow-brown,
Brucine. raspberry-red, brownish yellow,	Narcotine: blue-green, green, reddish yellow,
Codeine: greenish, upon warming blue,	Papaverine: violet-blue, then yellow,
Colchicin: yellow,	Solanine: yellow-red, red-brown, yellow,
Curarine: violet,	Thebaine: red, red-yellow,
Morphine: violet-red, then green,	Veratrine: yellow, orange, cherry-red.

The following give no color with Froehde's reagent: atropine, quinine, cocaine, caffeine, nicotine, strychnine, theobromine, cinchonine and cytisine.

¹ For the preparations of these reagents, see pages 641 to 643.

² Froehde's reagent does not keep long. Therefore it should be freshly prepared, or what is on hand should be tested with morphine to determine its fitness for use.

Mandeln's Reagent, or Vanadic-sulphuric Acid dissolves:

Berberine dirty green, later brown,	Narcotine· cinnabar-red to crimson-red,
Brucine red, yellow,	Narceine· violet, later red-yellow,
Emetine brown,	Papaverine blue-green, then blue,
Codeine green, upon warming blue,	Solamine orange-red, later violet,
Colchicin blue-green, green, brown,	Strychnine: blue-violet,
Curarine violet,	Thebaine orange-red,
Morphine red, then blue-violet,	Veratrine yellow, cherry-red

The following give no color with Mandeln's reagent: atropine, quinine, cocaine, caffeine, conune, nicotine, theobromine, cinchona and cytsine

Marquis¹ Reagent, or Formalin-sulphuric Acid dissolves:

Apomorphine· violet, rusty red, dark blue,
 Dionine pure blue,
 Heroine red, then blue-violet,
 Codeine reddish, blue-violet, violet-blue,
 Morphine peach-red, violet, blue-violet, pure blue,
 Narcotine: violet, olive-green, yellow,
 Papaverine wine-red, yellow, dirty brown-red, deep orange,
 Peronine red-violet,
 Veratrine yellow-brown, upon warming reddish brown

Mecke's² Reagent, or Selenious-sulphuric Acid dissolves:

	In the Cold	Upon Warming
Apomorphine	dark blue-violet,	dark brown,
Brucine	yellow-red,	lemon-yellow,
Codeine	blue, quickly emerald-green, later olive-green,	steel-blue, then brown,
Colchicin	lemon-yellow,	yellowish brown,
Morphine	blue, blue-green to olive-green,	brown,
Narceine·	greenish yellow, then violet,	dark violet,
Narcotine	greenish, steel-blue, then cherry-red,	cherry-red,
Papaverine·	greenish, dark steel-blue, then deep violet,	dark violet,
Physostigmine	brownish yellow,	pale brown-red,
Thebaine·	deep orange, gradually fading,	dark brown,
Veratrine·	lemon-yellow, olive-green	brownish violet.

¹ E. Marquis Distribution of Morphine in the Animal Body. Wiener mediz. Presse 1896 No 42 and Pharmaz Zentralhalle 37 (1896), 844

² A. Mecke A New Reagent for Alkaloids. Zeitschr. f. öffentl. Chem. 5 (1899), 350; Zeitschr. f. analyt. Chem. 39(1900), 468. See "Preparation of Reagents" page 643.

Mecke's reagent therefore is preeminently a sensitive reagent for most opium alkaloids. A great advantage of this reagent is the fact that it gives characteristic colors when alkaloids are not in a state of perfect purity.

Rosenthaler's¹ Reagent or Arsenic-Sulphuric Acid

Procedure.—Dissolve a small particle of the substance to be tested in 2-3 cc. of this reagent and warm the solution in a boiling water-bath. Fine colors that are very stable appear, if certain alkaloids are present. Usually these reactions are quite sensitive, giving distinct tests with 0.05 mg. of morphine, 0.02 mg. of narcotine, 0.1 mg. of codeine, dionine or apomorphine. A notable fact is that the alkaloids, hydrastine and hydrastinine, related to narcotine, also bear a close resemblance to this alkaloid in their behavior toward sulphuric acid containing arsenic acid. Of the other alkaloids only berberine and brucine give colors deserving mention but they are less distinct and not as fine.

	Cold	Warm	Warm, and after Addition of Hydro- chloric Acid
Codeine	light blue	dark blue	purple-red
Morphine	gradually greenish blue, then green	super-blue quickly emerald-green, later dark green	red-violet
Apomorphine:	super-yellow-green and green in blue	green	brown
Heroin:	yellow-brown with tinge of red	dark black-green	dark cherry-red
Dionine.	yellow	first blue, then green	purple-red, with a tinge of violet
Narcotine	greenish yellow	cherry-red	yellow-red
Hydrastine	yellow	cherry-red	yellow-red

Schaer's Reagent² or Perhydrol-Sulphuric Acid

Perhydrol, dissolved in concentrated sulphuric acid, gives fine color-reactions with various alkaloids. This reagent is prepared by carefully mixing 1 volume of 30 per cent. chemically pure "*Perhydrol*" with 10 volumes of pure concentrated sulphuric acid. This

¹ L. Rosenthaler and F. Türk. Sulphuric Acid containing Arsenic Acid as a Reagent for Alkaloids. Apoth.-Ztg. 19 (1904), 186

² E. Schaer: Reactions of Alkaloids with Perhydrol. Archiv d. Pharmaz. 248 (1910), 458.

reagent should always be freshly prepared. Use for each test 1 cc. of the cooled reagent and 5-10 mg. of the alkaloid. Quinine, or a quinine salt, gives a lemon-yellow to canary-yellow color. Strychnine in perhydrol-sulphuric acid, to which a small quantity of colloidal platinum solution has been added, gives slowly, usually not for several hours, a faint purple-red color that is characterized by great stability. This reagent, after previous addition of a little platinum solution, gives with brucine more of an orange-red, without platinum a reddish yellow. Morphine, codeine, narcotine, narceine and papaverine give with this perhydrol-reagent alone, or after addition of a little platinum solution, orange-red to purple-red, partly dark brown-yellow colors that soon disappear. Therefore these reactions are of little use in identifying opium alkaloids. Berberine gives a dark cherry-red with the perhydrol-reagent, gradually passing into brown-red. Hydrastine produces an intense chocolate-brown color that is especially distinct and comes quickly, if a little platinum solution has first been added to the reagent. According to Wasicky,¹ perhydrol-sulphuric acid also gives fine color-reactions with atropine, hyoscyamine and scopolamine. If a few drops of this reagent are poured upon a small quantity of one of these bases, the particles of the alkaloid as they pass into solution, that is, first at the edges, give an intense leaf-green color. After a few minutes this color passes into more of an olive-green and finally takes on a discolored brown-green appearance. Homatropine also gives this test. It also appears with cocaine but comes somewhat later and the color is more of an emerald-green.

Silicic-tungstic Acid or Godeffroy's Alkaloidal Precipitant

In 1876 Godeffroy² called attention to the great sensitiveness of silicic-dodecatungstic acid toward alkaloids and among others found that atropine was precipitated in a dilution of 1:15,000, quinine 1:50,000 and cinchonine 1:200,000 by this acid. Bertrand³ later subjected Godeffroy's reaction to a more critical examination.

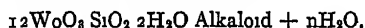
Silicic-tungstic acid, $12\text{WoO}_3 \cdot \text{SiO}_2 \cdot 2\text{H}_2\text{O}$, forms perfectly stable, well-defined salts. In 5 per cent. aqueous solution it may be used as such or as an alkali salt. The sensitiveness of the reaction exceeds nearly all the general tests for alkaloids.

¹ R. Wasicky: A New and Very Sensitive Color-reaction of Atropine, Hyoscyamine and Scopolamine. *Zeitschr. f. analyt. Chem.* 54 (1915), 393.

² R. Godeffroy: Use of Silicic-Dodeca-Tungstic Acid as a Reagent for Alkaloids. *Ber. d. Deutsch. chem. Ges.* 9 (1876), 1792.

³ G. Bertrand: *Compt. rend.* 128,742 and *Bull. de la Soc. chim. de Paris* (3 série) 21 (1903), 434.

thus far recommended With cold sufficiently concentrated solutions of alkaloidal salts this reagent usually produces precipitates that are flocculent, gather into balls, are pulverulent, or even crystalline They are white, faintly yellowish or reddish in color and can easily be filtered from the liquid These precipitates are almost insoluble in cold water and but slightly soluble at boiling temperature. In composition they have the general formula.



These silico-tungstates are insoluble even in concentrated acids and upon ignition give a residue consisting of $12\text{WoO}_3 \cdot \text{SiO}_2$

Morphine silico-tungstate, $12\text{WoO}_3 \cdot \text{SiO}_2 \cdot 2\text{H}_2\text{O} \cdot 4\text{C}_{17}\text{H}_{19}\text{NO}_3 + 9\text{H}_2\text{O}$, is amorphous, salmon-colored and contains, when dried at 120° , $2\text{H}_2\text{O}$

Strychnine silico-tungstate, $12\text{WoO}_3 \cdot \text{SiO}_2 \cdot 2\text{H}_2\text{O} \cdot 4\text{C}_{21}\text{H}_{27}\text{N}_3\text{O}_2 + 8\text{H}_2\text{O}$, is similar in appearance to the morphine salt and contains $1\text{H}_2\text{O}$ when dried at 120° .

If after precipitation liquid and precipitate are heated together, the latter loses a certain quantity of water and then forms a finely divided powder that settles rather slowly In the case of various alkaloids the precipitate in this condition is more distinctly visible than when precipitated cold.

In establishing the limit of delicacy, Bertrand added for each 5 cc. of alkaloidal salt solution 1-2 drops of the silicic-tungstic acid reagent and the same quantity of 10 per cent hydrochloric acid He found the limit for cocaine 1:8000, nicotine 1:20,000, narceine 1:30,000, codeine 1:40,000, atropine and caffeine 1:50,000, and cocaine 1:200,000 It made no difference with these alkaloids whether they were precipitated from hot or cold solution The sensitiveness of the following bases was much greater, if they were first heated to boiling and then allowed to cool, and was found to be for aconitine 1:80,000, veratrine 1:130,000, brucine 1:150,000, strychnine and narcotine 1:200,000, and quinine and cinchonine 1:500,000

Alkaloidal silico-tungstates are easily attacked by oxidizing agents. This fact makes it possible to use these precipitates for those characteristic color-reactions that depend upon processes of oxidation, for example, the test for strychnine with sulphuric acid and potassium dichromate

Alkaloidal silico-tungstates are at once decomposed even in the cold by dilute aqueous solutions of caustic alkalies and also by ammonia In this way the free alkaloidal bases can easily be separated from these precipitates They may then either be filtered off, or better extracted by an appropriate solvent, such as ether or chloroform. In this procedure silicic-tungstic acid remains in the aqueous alkaline liquid as a salt

CHAPTER III

METALLIC POISONS

Fresenius-v. Babo¹ Procedure for Destruction of Organic Matter

The residue left after removal of volatile poisons by steam-distillation may be used in this part of the analysis, as it must contain poisonous metals if any are present. A portion of the original material,² previously finely chopped and well-mixed with enough water to produce a fluid mass, may also be used. According to the quantity of material to be destroyed, add 10-15 cc of pure concentrated hydrochloric acid and 1-2 grams of potassium chlorate. Set the flask upon a boiling water-bath in a good draught, heat and shake frequently so that chlorine comes into intimate contact with material to be destroyed. When the mixture is hot, add 0.3-0.5 gram of potassium chlorate every 2-3 minutes and shake the flask frequently. Continue in this manner until most of the organic matter is dissolved and the liquid in the flask is clear, or turbid, and has a wine-yellow color. Further addition of potassium chlorate and longer heating should produce no change. Fat and fatty acids in particular are extremely resistant to the action of chlorine.

When organic matter is completely destroyed, dilute with hot water, adding a few drops of dilute sulphuric acid to precipitate possible barium, shake and pour the cold liquid through a moistened filter. If the excess of free hydrochloric acid is not too large, the filtrate may be saturated direct with hydrogen sulphide. Otherwise, evaporate the solution in a porcelain dish upon the water-bath nearly to dryness to remove most of the free hydrochloric acid. This step

¹ R. Fresenius and L. v. Babo. A New Method dependable under all Conditions for Detection and Quantitative Estimation of Arsenic in Cases of Poisoning. *Annalen d. Chem. u. Pharm.* 49 (1844), 287

² Cadaveric material should be divided as finely as possible, then brought to a thin mixture by stirring with 12.5 per cent arsenic-free hydrochloric acid, and heated with frequent shaking with 1-2 grams of potassium chlorate as directed above. If the material is heated upon the water-bath in a porcelain dish, it should be stirred constantly.

frequently produces a dark color which a few crystals of potassium chlorate will discharge.

An alternative procedure is to remove part of the free hydrochloric acid from the filtrate, obtained after treatment with hydrochloric acid and potassium chlorate, by first evaporating to a smaller volume and then adding ammonia until alkaline. Finally add dilute nitric acid until the solution is faintly acid. Then saturate the liquid, which should not contain too much free acid, with arsenic-free hydrogen sulphide as directed upon page 246

The insoluble residue upon the filter, obtained by heating the distillation-residue, or the original material, with hydrochloric acid and potassium chlorate, may contain silver chloride, barium sulphate and lead sulphate in addition to fat. Examine as directed under "Metallic Poisons IV" for silver, barium and lead (see page 262).

Thoms¹ destroys organic matter in the apparatus shown in Fig. 17. Oxidation is carried on in an ordinary fractioning flask (A) with the tubulus (B) bent upward. A separating funnel (C), held in the neck

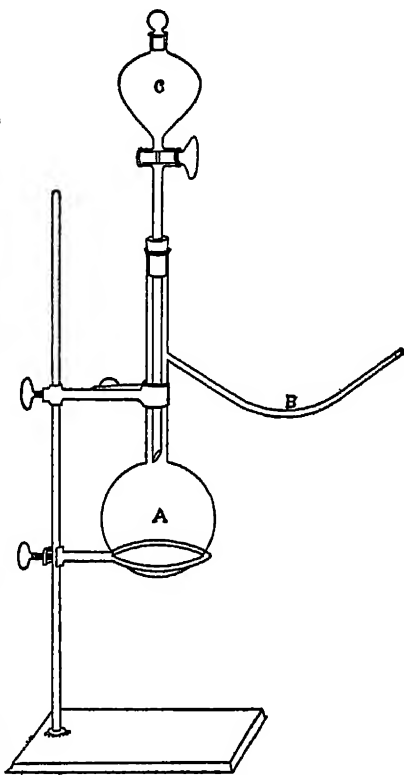


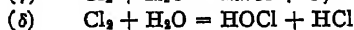
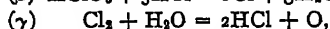
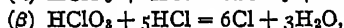
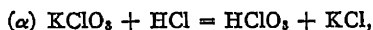
FIG. 17.—Thoms flask for destroying organic matter.

of the flask by a stopper, contains an aqueous solution of potassium chlorate (1:20) saturated at ordinary temperature. Organic matter, for example, finely divided organs, or other cadaveric material, is in the flask as a thin mixture with 12.5 per cent. hydrochloric acid. Add about 1 gram of solid potassium chlorate and warm the flask upon a boiling water-bath. When the mass in the

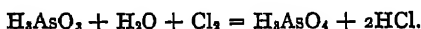
¹ H. Thoms. Introduction to the Practical Chemistry of Foods. Published by S Hirzel, Leipsic, 64 illustrations and 163 pages.

flask is warm, allow the potassium chlorate solution to run in drop by drop and shake constantly. Care must be taken not to add much of this solution at once, otherwise explosions may easily occur from formation of chlorine dioxide (ClO_2). In other respects procedure is identical with that described above.

Notes.—Potassium chlorate and hydrochloric acid evolve chlorine (α and part of which acts upon the organic material destroying it, and part in presence of water forms oxygen and oxygen-acids of chlorine (γ and δ) which are strong oxidizing agents.

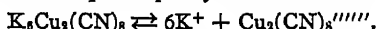


Arsenious acid, present in the material, probably is not volatilized as arsenic trichloride (AsCl_3) by the procedure described but oxidized to non-volatile arsenic acid

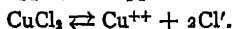


Even after most thorough treatment of parts of the cadaver, or vegetable substances, with hydrochloric acid and potassium chlorate, a more or less abundant white precipitate, consisting in part at least of fat and free fatty acids (chlorinated fatty acids) and exceedingly resistant to the action of nascent chlorine, always remains. This treatment converts a portion of the organic matter into volatile compounds (chloranil?) which have a sharp odor and attack the mucous membranes. For this reason destruction of organic material must take place in a hood with a good draught.

Treatment with hydrochloric acid and potassium chlorate, as described, converts metallic poisons into inorganic salts, usually chlorides and sulphates, which either remain in solution, or are precipitated as difficultly soluble silver chloride, barium sulphate, and in part also as lead sulphate. Protein substances, present in all animal and vegetable organisms and fluids, precipitate many heavy metals such as mercury, silver, lead, copper, uranium and zinc, from solutions of their salts. These metals are then in the form of metallic albuminates, which usually dissolve in water with great difficulty and are very stable. As a rule these metallic protein compounds must receive further treatment before it is possible to detect the metal. Many organic acids, such as tartaric acid and carbohydrates, interfere more or less with the detection of heavy metals. In combination with these organic substances, heavy metals behave like copper in potassium cuprocyanide, $\text{K}_4\text{Cu}_2(\text{CN})_6$, which is precipitated by neither sodium hydroxide nor hydrogen sulphide, because in solution it is electrolytically dissociated, as shown in the equation, into the complex cupro-cyanide ion and not into copper ion



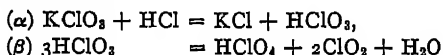
If potassium cupro-cyanide is heated with hydrochloric acid and potassium chlorate, copper passes into solution as cupric chloride. The reagents mentioned above will then precipitate copper, for copper chloride is ionized as follows:



Consequently the solution of cupric chloride will give the reactions of copper

If metallic poisons are to be detected by ordinary ionic reactions, interfering organic substances, especially protein bodies, must be removed, that is, destroyed by some procedure whereby metals are converted into inorganic salts that undergo ionization

Potassium chlorate acts best upon organic substances in strong hydrochloric acid solution. If the mass becomes too thick at any time during heating, it should be diluted with water or dilute hydrochloric acid. The contents of the flask should also be well-shaken during treatment with potassium chlorate, to prevent collection of a large quantity of this salt upon the bottom of the flask. Such an occurrence may cause an explosion due to formation of the exceedingly unstable chlorine dioxide (ClO_2)



In examining cadaveric material, the author employs 12.5 per cent. hydrochloric acid (sp. gr. 1.061) saturated with hydrogen sulphide and kept in a loosely stoppered bottle. This insures precipitation of the final traces of arsenic sometimes present even in the purest commercial acid.¹ When needed, this acid is filtered through ash-free paper to remove precipitated sulphur that may contain arsenic sulphide.

Cadaveric material is destroyed by the mixture of hydrochloric acid and potassium chlorate with relative rapidity and for the most part passes into solution. An experiment, in which 100 grams of stomach and duodenum, 20 grams of stomach-contents, 75 grams of kidney, and 200 grams of liver (395 grams in all) were treated as described, required about 1 hour for complete solution. The insoluble part, filtered, washed and dried upon a porous earthen plate, weighed 52 grams, and 32 grams when dried at 100°. It was an amorphous, yellowish white, fatty mass and almost entirely soluble in hot sodium hydroxide solution.

Vintilesco's² Modification of Fresenius-v. Babo Method of Destroying Organic Matter

After a critical discussion of the methods of Gautier, Denigès, Meillère, Kerbosch and Breteau, Vintilesco recommends the potassium chlorate method as best for destruction of organic substances in toxicological examinations, especially when carried out in the following manner:

Mix 200 grams of finely comminuted material in a porcelain dish with 10 per cent. of its weight of potassium chlorate, and heat this mixture upon a water-bath until evolution of ammonia has ceased. Then stir continuously and add pure concentrated hydrochloric acid (sp. gr. 1.19), 5 cc. every 5-10 minutes (25-40 cc. in all), and continue heating until evolution of chlorine has ceased. This usually requires about 40 minutes. Now add to the mixture 10-20 cc. of pure nitric acid of 40° B ϕ (62-63 per cent. HNO_3). Stir from time to time and allow the

¹ Use of electrolytic hydrochloric acid (see page 500) avoids the necessity of this purification. Tr.

² J. Vintilesco. Observations upon Some Methods of Destroying Organic Matter in the Detection of Metallic Poisons. Choice of a General Analytical Method. Bull. de Chim. 17 (1915), 99.

dish to remain upon the water-bath until most of the acid has evaporated. By this procedure only about 10 per cent. of organic material is not destroyed, that is, undissolved, consisting mostly of fat and fatty acids. The filtered liquid usually is yellowish and is excellently adapted for detection of metallic poisons by the general methods of analysis.

Destruction of Organic Matter by Free Chloric Acid

Sonnenschein and Jeserich¹ use pure chloric acid instead of potassium chlorate. Stir the finely divided material with water until the mass is thin and then transfer to a large flask together with a few cc. of chloric acid. Warm slowly and cautiously upon the water-bath. As soon as the mass swells and becomes porous, gradually add small portions of hydrochloric acid. Even a considerable quantity of cadaveric material will dissolve in 2-3 hours. Complete destruction of organic matter may be recognized by the fact that the upper layer of fatty acids is almost white and the liquid below nearly clear and yellowish. Water lost by evaporation should be replaced from time to time, otherwise the reaction may proceed with explosive violence. In other respects the mixture should be treated as in the procedure with potassium chlorate and hydrochloric acid.

Mar's² Method of Destroying Organic Matter

Mix finely divided material with dilute hydrochloric acid (1:12) until thin. Add a little potassium chlorate and heat over a free flame, adding from time to time small quantities of potassium chlorate (0.2 gram). Cool as soon as liquefaction of the mass is complete. Fat separates and usually can be easily removed from the liquid. Heat this fat once or twice with very dilute nitric acid, filter and add the filtrate to the main part of the liquid. Continue heating the latter adding small quantities of ammonium persulphate, until the liquid is clear and yellowish white. Filter and saturate the filtrate as usual with hydrogen sulphide.

Ammonium persulphate is a powerful oxidizing agent which upon evaporation of its aqueous solution is decomposed into ammonium sulphate, sulphuric acid and oxygen.



As compared with many other oxidizing agents, it possesses the great advantage of not adding non-volatile substances to the material under examination.

Other methods of destroying organic matter are given in Chapter V of this book (see pages 486 to 514).

EXAMINATION OF FILTRATE FOR METALLIC POISON

Precipitation by Hydrogen Sulphide

A solution properly prepared by the Fresenius-v. Babo or by any other method, freed from excess of hydrochloric acid and filtered.

¹ P. Jeserich. *Repertorium der analyt. Chem.* 2 (1882), 379.

² C. Mai. *Critical Operations in the Field of Forensic Chemistry.* *Zeitschr. Unters. d. Nahrgs.-u. Genussm. usw.* 5 (1902), 1106.

should have only a faint yellow color ¹ Heat such a solution in a flask upon the water-bath and saturate with arsenic-free hydrogen sulphide ² Pass hydrogen sulphide for 0.5-1 hour, or longer, into the hot solution and continue this treatment after the solution has been removed from the water-bath and is cold.³

Allow the solution saturated with hydrogen sulphide to stand in the loosely stoppered flask for several hours, or better until the

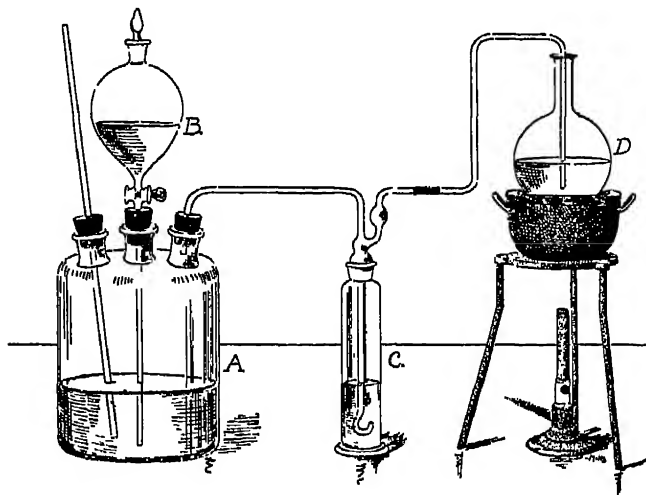


FIG. 18.—Apparatus for generating arsenic-free hydrogen sulphide (a) Generator with dilute sulphuric acid, (b) Separating funnel with NaSH, (c) Wash-bottle, (d) Hydrochloric acid solution of material.

following day. If the solution then smells of hydrogen sulphide and blackens a piece of lead paper held over it, the next step in the process may be taken. Otherwise, warm the solution once more

¹ Chromium in not too small quantity imparts more or less of a green color both to solution and filtrate from the hydrogen sulphide precipitate, owing to presence of chromic chloride (CrCl_3).

² Prepare arsenic-free hydrogen sulphide by saturating dilute sodium hydroxide solution with hydrogen sulphide from crude iron sulphide and commercial hydrochloric acid. Pour this sodium hydro-sulphide (NaSH) solution into a separating funnel and add slowly to dilute sulphuric acid (1.4). Hydrogen sulphide is given off at once and can be regulated at will. It is practically arsenic-free and is especially suited to most forensic cases. The apparatus required is shown above in Fig. 18.

³ In laboratory experiments, treatment with hydrogen sulphide may be somewhat shortened. A Kipp generator in which the gas is prepared from iron sulphide and hydrochloric acid may be used.

upon the water-bath and again saturate with hydrogen sulphide. Finally collect the hydrogen sulphide precipitate upon a sm. paper and wash with hydrogen sulphide water.

Examine the precipitate, produced by hydrogen sulphide, for arsenic, antimony, tin, mercury, lead, copper, bismuth and cadmium (Metallic Poisons I and II), and the filtrate from this precipitate for chromium and zinc (Metallic Poisons III, page 260).

Notes.—The fact should not be overlooked that vegetable and animal substances, consequently cadaveric material, when treated with hydrochloric acid and potassium chlorate, frequently give liquids, yielding yellow-red, brown- or dark brown precipitates¹ with hydrogen sulphide even in absence of metal of this group. Such precipitates probably consist in part of organic sulphur compounds. Consequently in toxicological examinations, if hydrogen sulphide produces such a colored precipitate in acid solution, it should never be interpreted to mean that a metallic poison is positively present. Moreover, without further examination it is impossible to decide from the color of the hydrogen sulphide precipitate as to the presence of a particular metal.

Complete Precipitation.—Before testing for chromium and zinc in the filtrate from the hydrogen sulphide precipitate, add about five times the volume of strong hydrogen sulphide water to a portion of the filtrate, shake and allow to stand for several minutes. Until a colored precipitate appears, the metals in question (Metallic Poisons I and II) have been completely removed, and the filtrate may then be further tested for chromium and zinc (Metallic Poisons I and II). Otherwise, first strongly dilute the entire filtrate from the hydrogen sulphide precipitate with water and again saturate with hydrogen sulphide. In presence of much hydrochloric acid, lead and cadmium are incompletely precipitated by hydrogen sulphide.

Extraction of Hydrogen Sulphide Precipitate with Ammonium Ammonium Sulphide

Thoroughly extract the washed hydrogen sulphide precipitate while moist, upon the filter with a hot mixture of approximately equal parts of ammonia and yellow ammonium sulphide. This may be accomplished by heating 5–10 cc. of the mixture of ammonia and ammonium sulphide to boiling and dropping the solution over

¹ Repeated treatment with potassium chlorate and hydrochloric acid dissolves thoroughly washed casein and fibrin almost completely, giving a filtrate in which hydrogen sulphide precipitates dirty yellow to brownish substance. Such precipitates are amorphous and contain together with much free sulphur organic sulphur compounds, possibly thio-aldehydes and ketones.

precipitate upon the filter. Reheat the filtrate and pour again over the precipitate. Repeat this operation several times. Finally wash the filter with a few cc. of fresh ammonia-ammonium sulphide mixture.

Test the entire filtrate for arsenic, antimony, tin and copper¹ (Metallic Poisons I) and the residue left upon the filter for mercury, lead, copper, bismuth and cadmium (Metallic Poisons II).

METALLIC POISONS I

Arsenic, Antimony, Tin, Copper

Examination of Hydrogen Sulphide Precipitate Soluble in Ammonia-Ammonium Sulphide

Use the solution prepared as described by treating the hydrogen sulphide precipitate with a hot mixture of ammonia and yellow ammonium sulphide. This solution is usually dark brown owing to dissolved organic substances.² Evaporate the solution to dryness in a porcelain dish upon the water-bath. Moisten the cold residue with fuming nitric acid and again evaporate. Then intimately mix the residue with about three times the quantity³ of a mixture of 2 parts of sodium nitrate and 1 part of dry sodium carbonate. Thoroughly dry this mixture upon the water-bath and introduce small portions at a time into a porcelain crucible containing a little fused sodium nitrate heated to redness. After the final addition, heat the

¹ Copper sulphide (CuS) is soluble to considerable extent in hot yellow ammonium sulphide. An ammonium sulphide solution containing copper, treated as described on page 250, yields copper oxide which gives the melt a more or less gray or black appearance. If the melt is extracted with water, the residue contains black copper oxide with stannic oxide and sodium pyroantimonate. To detect copper, dissolve the black residue in a little hot dilute hydrochloric acid and divide the solution into two parts. Add ammonia to one part until alkaline. The solution is blue, if copper is present. Add potassium ferrocyanide to the other part. A brownish red precipitate of cupric ferrocyanide ($\text{Cu}_2\text{Fe}(\text{CN})_6$) will appear, if copper is present.

² In toxicological examinations, even in absence of metals, hydrogen sulphide produces in the hydrochloric acid solution in most cases dark colored precipitates (see above). The latter are due to organic substances always present and usually soluble with a deep dark brown color in the hot ammonia-ammonium sulphide mixture.

³ In most laboratory experiments, 3 grams of a mixture of 2 grams of sodium nitrate and 1 gram of sodium carbonate are sufficient. Too great an excess of sodium nitrate should be avoided.

crucible a short time, introducing possibly a little more sodium nitrate, until the fused mass is colorless. In presence of copper the melt is gray, or grayish black, from copper oxide. Sodium arsenate, sodium pyro-antimonate, sodium stannate, as well as stannic oxide and copper oxide, may also be present. Soften the cold melt with hot water and wash into a flask. Add a little sodium bicarbonate to the clear or cloudy liquid, to decompose the small quantity of sodium stannate, possibly in solution, and precipitate all the tin as stannic oxide and filter.

Any arsenic present will be in the filtrate (A) as sodium arsenate (Na_2HAsO_4) and the residue upon the filter (B) may contain antimony as sodium pyro-antimonate ($\text{Na}_2\text{H}_2\text{Sb}_2\text{O}_7$), tin as the oxide (SnO_2), as well as copper oxide.¹

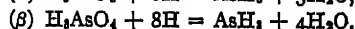
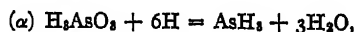
Examination of Filtrate A for Arsenic

In toxicological examinations proof of the presence of arsenic in general is afforded by isolating this poison in its elementary condition and thereby removing all doubt as to its existence in the given material. Two methods are in use for this purpose, namely, the Berzelius-Marsh and the Fresenius-v. Babo method. Both are very accurate and exclude all confusion of arsenic and antimony.

Chapter V of this book gives the details of the very delicate biological test for detecting arsenic, requiring the use of certain moulds; the electrolytic separation of arsenic at the cathode as arsine; and the arsenic tests of Bettendorff, Gutzeit and Reinsch. In the same part of this book, isolation of arsenic as trichloride by the Schneider-Fyfe-Beckurts method and its colorimetric estimation have been taken up.

1. Berzelius-Marsh Method

Principle.—Nascent hydrogen converts oxygen compounds of arsenic, arsenious and arsenic acid, as well as their salts into arsine (AsH_3):



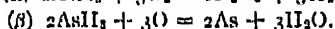
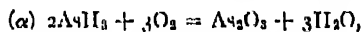
At red heat arsine is decomposed into metallic arsenic and hydrogen:



This equation expresses formation of the arsenic mirror.

¹ Even in absence of the substances mentioned under B, a small insoluble residue usually appears. This may come from the porcelain crucible, the glazing of which is slightly attacked in the fusion with sodium nitrate and carbonate.

Hydrogen containing arsine burns with a bluish white flame to arsenic trioxide and water (α). Depress a piece of cold porcelain upon this flame. Only hydrogen burns, whereas arsenic is deposited as the metal (β). This is the so-called arsenic spot:



Hydrogen containing arsine, passed into dilute silver nitrate solution, precipitates black metallic silver. At the same time arsenious acid passes into solution:



Procedure.—Acidify the solution of the melt, that is, "Filtrate A," prepared as described above (page 250) and possibly containing sodium arsenate, with dilute arsenic-free sulphuric acid. Evaporate this solution in a porcelain dish upon an asbestos plate over a small flame. Add 15–20 drops of concentrated sulphuric acid, to expel completely any nitric acid present in the residue, and heat until copious white fumes of sulphuric acid are given off. The residue in the dish is a thick, colorless liquid having a strong acid reaction. Arsenic, if present, is in the form of arsenic acid (H_3AsO_4) and when cold frequently solidifies to a white crystalline mass. Examine the solution of this residue for arsenic in the Marsh apparatus. The solution is also well adapted for the electrolytic detection of arsenic (see pages 518 and 524).

Marsh Apparatus.—Place 15–25 grams of pure arsenic-free zinc (granulated or in small rods) in the reduction-flask A of the Marsh apparatus (Fig. 19). Pour cold dilute arsenic-free sulphuric acid through the thistle-tube upon the zinc. This acid should contain 15–16 per cent. of H_2SO_4 .¹ Evolution of hydrogen should not be too rapid, otherwise the liquid in the evolution-flask may get too warm. The result may be reduction of some sulphuric acid to sul-

¹ To insure complete removal of nitric acid, test a few drops of this residue by mixing with concentrated sulphuric acid and carefully adding to a cold saturated solution of ferrous sulphate.

² The passage of hydrogen from 15–20 grams of zinc, treated with dilute arsenic-free sulphuric acid, through the strongly heated ignition-tube C of the Marsh apparatus should not give a trace of arsenic after 1 hour. Such zinc is practically arsenic-free and is suited for forensic-chemical examinations. Best spelter from the New Jersey Zinc Company will meet this test.

³ Add 1 volume of pure arsenic-free concentrated sulphuric acid to 5 volume of distilled water. This acid when cold is suitable for use in the Marsh test.

phurous acid and even to hydrogen sulphide, more or less interfering with or entirely preventing detection of arsenic. Therefore place reduction-flask A in a dish of cold water, if the acid becomes too warm. In using the Marsh apparatus, the following precautions should be taken:

1. Make sure that the apparatus is absolutely tight
2. Expel air completely before igniting hydrogen. To tell when this point is reached, collect hydrogen in a dry test-tube until it ignites without detonation when carried to a flame. When hydrogen stands this test, ignite the gas at the end of ignition-tube C.

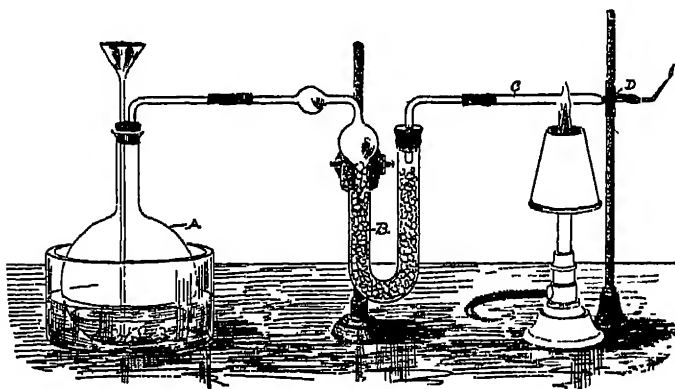


FIG 19—Marsh apparatus for the detection of arsenic and antimony (a) Hydrogen generator; (b) Calcium chloride drying-tube, (c) Hard glass tube, (d) Arsenic mirror

There is then no danger of an explosion within the apparatus. If the apparatus is tight and evolution of hydrogen is not too rapid, it requires about 5–8 minutes to expel the air.

3 Test hydrogen to insure entire freedom from arsenic. Neither arsenic mirror nor spot should appear.

If hydrogen is arsenic-free, gradually introduce the perfectly cold sulphuric acid solution, containing arsenic as arsenic acid, in small portions into reduction-flask A. At the same time heat ignition-tube C to redness just back of the capillary tube. If the solution contains arsenic, the gas generated consists of a mixture of arsine (AsH_3) and hydrogen. A shining mirror of metallic arsenic appears, often in a few minutes, just beyond the point of ignition. Traces of arsenic require considerable time before a brown or brown-

ish black film appears. A piece of white paper held behind the tube brings out clearly even a minute arsenic mirror.

Remove the flame from ignition-tube C. If arsenic is present, the hydrogen flame will become bluish white. At the same time white fumes of arsenic trioxide (As_2O_3) will arise from the flame

To produce the lustrous, brownish black spot (arsenic spot), depress a cold porcelain dish upon the hydrogen flame.

Arsine has an exceedingly characteristic, garlic-like odor. Extinguish the hydrogen flame and allow the gas to escape.¹ The odor is evident even when hydrogen contains only traces of arsine.

A third method of detecting arsenic by the Marsh apparatus consists in extinguishing the hydrogen flame and passing the gas into dilute neutral silver nitrate solution. Arsine will darken this solution, producing a black precipitate of metallic silver. The solution contains arsenious acid and free nitric acid.

Filter through a double paper to remove silver and carefully neutralize the filtrate with a few drops of very dilute ammonium hydroxide solution. If the solution is neutral, it is possible to obtain a yellowish white precipitate of silver arsenite (Ag_3AsO_3) but this compound dissolves easily in ammonium hydroxide solution and in nitric acid.

Gutzeit's Arsenic Test.—Extinguish the flame at the end of reduction-tube C and hold over the tube a strip of paper moistened with concentrated silver nitrate solution (1:1). A yellow stain will appear, if hydrogen contains arsine. A drop of water added to this yellow spot will turn it black.

In many cases it is advisable to pass through the Marsh apparatus during the entire test a moderate stream of hydrogen generated in a Kipp apparatus from arsenic-free zinc and dilute sulphuric acid. The gas should also be washed through water before it enters the Marsh apparatus. The arrangement of such an apparatus is shown in Fig. 20.

Differences between Spots and Mirrors of Arsenic and Antimony

Nascent hydrogen reduces various antimony compounds (SbCl_3 , Sb_2O_3 , HSbO_3 , $\text{KSbOC}_4\text{H}_4\text{O}_6$, etc.), producing the colorless gas stibine (SbH_3). The behavior of this compound in the Marsh apparatus closely resembles that of arsine, for it gives a spot and mirror but produces a black precipitate of silver

¹ Detection of arsine is so easy by other tests that it seems somewhat superfluous to confirm its presence in this way in view of its very poisonous properties.
Tr.

4 Gently heat the ignition-tube and pass a stream of dry hydrogen sulphide over the arsenic mirror. Yellow arsenic trisulphide (As_2S_3) will appear. The antimony mirror becomes brown-red to black (Sb_2S_3).

5 Arsine and stibine are differentiated by their behavior toward dilute silver nitrate solution. Both produce black precipitates. Arsine precipitates metallic silver and the filtrate contains arsenious acid. On the other hand, stibine precipitates silver antimonide (Ag_3Sb) and the filtrate does not contain a trace of antimony. To detect antimony, filter off the black precipitate, wash and boil for some time with 10-15 per cent tartaric acid solution. Antimony is completely dissolved, whereas silver remains as a grayish white residue. Add dilute hydrochloric acid to this solution and then treat with hydrogen sulphide. Antimony will appear as orange-red antimony sulphide.

2. Fresenius-von Babo Method

Principle.—Fusion of oxygen and sulphur compounds of arsenic with a mixture of sodium carbonate and potassium cyanide causes reduction with formation of an arsenic mirror. As a result potassium cyanide is converted into potassium cyanate or sulphocyanate.



Procedure.—Use for this test the aqueous, sulphuric acid solution (A), prepared by the method already described and containing arsenic in the form of

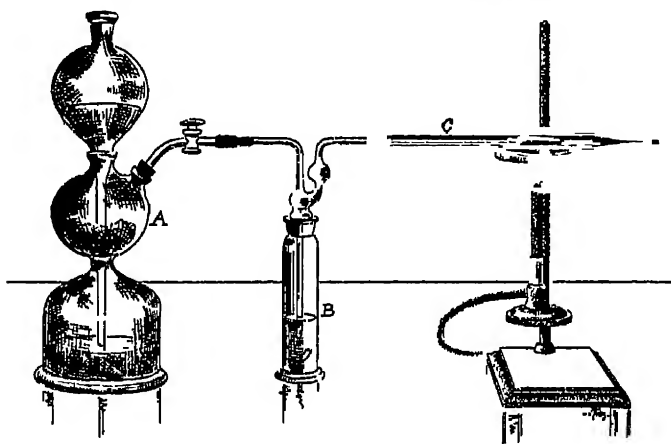


FIG 21.—Fresenius-von Babo apparatus (a) Carbon dioxide generator; (b) Drying-bottle with pure, concentrated sulphuric acid, (c) Ignition-tube and boat.

arsenic acid (page 251). To reduce arsenic acid to arsenious acid, add a few cc of sulphurous acid to the solution and heat until odor of this acid has entirely disappeared. Dilute this solution with water and treat with hydrogen sulphide. Collect the precipitate (As_2S_3) upon a small filter and wash thoroughly. Dis-

antimonide (Ag_3Sb) instead of metallic silver, when passed into silver nitrate solution

Although the procedure described separates arsenic from antimony and excludes possibility of these two metals appearing together in the Marsh test, it seems advisable to point out the differences between them, especially as it is always necessary to identify arsenic mirrors and spots by other tests. In many cases, particularly when tests have indicated presence of antimony, the mirror and spot of this metal should be produced in the Marsh apparatus with the liquid suspected to contain antimony, for the purpose of confirming the other tests.

Differences between spots and mirrors of arsenic and antimony are as follows:

1. The arsenic mirror has a high metallic luster. It is brownish black and easily volatile. Owing to the latter property, it sublimes when heated in a stream of hydrogen. In case of the antimony mirror, which appears on both sides of the flame, the metal in contact with the heated glass fuses and is silver-

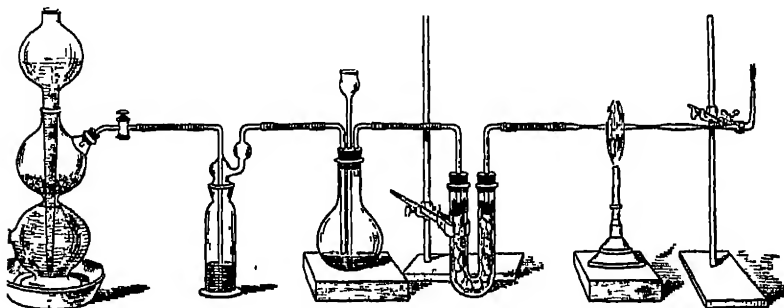
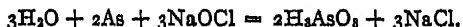


FIG. 20.—Marsh apparatus combined with Kipp generator.

white. But in places removed from the flame it is almost black and has hardly any luster. Stibine decomposes at a temperature much below that required for arsine. This fact explains deposition of this metal on both sides of the flame. Antimony volatilizes at a high temperature and consequently sublimes with difficulty.

2. The arsenic spot, if not too heavy, is brownish black or brown and lustrous. It dissolves readily in sodium hypochlorite solution, forming arsenious and arsenic acid.



The antimony spot is dull, velvet-black and without luster. A thin film of antimony is never brown but has a dark, graphite-like appearance. It is insoluble in sodium hypochlorite solution.

3. A drop of concentrated nitric acid, or moist chlorine, at once dissolves the arsenic spot forming arsenic acid. Neutralize with ammonia and add silver nitrate solution. A reddish precipitate of silver arsenate (Ag_3AsO_4) will appear.

Nitric acid, or moist chlorine, also dissolves the antimony spot but silver nitrate does not produce a colored precipitate.

solve the precipitate upon the filter in a little hot ammonium hydroxide solution. Evaporate this solution in a porcelain dish upon the water-bath and heat the residue with concentrated nitric acid. Expel the latter completely by evaporation, moisten the residue with a little water, and add enough dry sodium carbonate to render the mixture distinctly alkaline. Dry thoroughly upon the water-bath and triturate the residue in a mortar with several times the quantity of a mixture of 3 parts of dry sodium carbonate and 1 part of pure potassium cyanide.

Transfer this mixture to a porcelain boat and place in an ignition-tube of hard glass. Heat in a stream of carbon dioxide dried by means of arsenic-free sulphuric acid (Fig 21). To expel moisture, first heat the ignition-tube gently where the boat is and then ignite at a bright red heat. A mirror of arsenic will appear upon the cooler part of the tube, if arsenic is present.

A simpler method of detecting arsenic by potassium cyanide is often used. Heat the thoroughly dried material containing arsenic (As_2O_3 , As_2S_3) in a bulb-tube with a dry mixture of sodium carbonate and potassium cyanide until fusion takes place. If the tube is smaller above the bulb, the arsenic mirror will form in the constricted area (Fig 22).

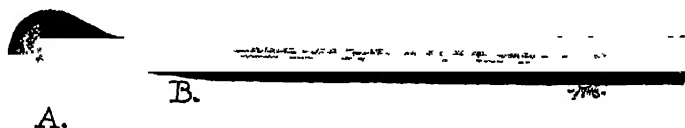


FIG 22 —(A) Substance and fusion-mixture, (B) Arsenic mirror.

Examination of Residue B for Antimony, Tin and Copper

Residue B (see page 250), insoluble in water and obtained by fusion, may contain sodium pyro-antimonate, stannic and cupric oxide. Treat this residue upon the filter with a little hot dilute hydrochloric acid (equal parts of concentrated acid and water). Pass this acid repeatedly through the paper until most of the residue is dissolved. If the original color of residue B and melt was gray or black, first examine a portion of the hydrochloric acid solution for copper. Excess of ammonia will produce a blue color. Potassium ferrocyanide solution will give a brownish red precipitate, or only a coloration with traces of copper.

Concentrate the remainder of the hydrochloric acid solution to a few drops in a porcelain dish upon the water-bath, and put two drops of this solution upon platinum foil in contact with zinc. Antimony will produce a black, tin a grayish and copper a dark reddish brown spot upon platinum. There is little chance of confusing the tin or copper spot with that given by antimony.

Dilute the remainder of the hydrochloric acid solution of residue B with water and introduce a piece of zinc. Keep the zinc in the solution as long as hydrogen is evolved. Collect black, metallic flocks from this operation upon a small filter. Wash thoroughly and gently warm with a little concentrated hydrochloric acid. Finally filter the solution. Antimony does not dissolve, whereas tin passes into solution as stannous chloride (SnCl_2) and is in the filtrate. It may be recognized by the following tests:

(a) **Mercury Test.**—Add a few drops of mercuric chloride solution to a portion of the filtrate. Tin will precipitate white mercurous chloride. In addition, heat will produce gray, metallic mercury, if stannous chloride is present in large excess.

(b) **Prussian Blue Test.**—Add a few drops of a dilute mixture of ferric chloride and potassium ferricyanide solution to a second portion of the filtrate. Tin will produce a precipitate of Prussian blue.

This test is not characteristic of tin, for many other substances capable of reducing ferri-ferricyanide to ferri-ferrocyanide, that is, to Prussian blue, act in the same way

For further identification of antimony, dissolve black flocks, insoluble in hydrochloric acid, in a few drops of hot aqua regia. Expel excess of acid upon the water-bath and dilute the residue with water. If the quantity of antimony is not too small, water will precipitate white antimony oxychloride (SbOCl). Redissolve this precipitate in a little dilute hydrochloric acid. Test a portion of this solution for antimony with hydrogen sulphide. Introduce the remainder into the Marsh apparatus and produce the antimony spot and murret, or test for stibine with silver nitrate solution as described (see page 255).

METALLIC POISONS II

Mercury, Lead, Copper, Bismuth, Cadmium

Examination of Hydrogen Sulphide Precipitate Insoluble in Ammonium Sulphide

That portion of the hydrogen sulphide precipitate, insoluble in ammonium sulphide, may contain sulphides of mercury, lead, copper, bismuth and cadmium. Examine this precipitate according to the general methods employed in qualitative analysis. If there is only a slight precipitate, treat it repeatedly upon the filter with a few cc.

of warm, rather dilute nitric acid (1 volume of concentrated acid and 2 volumes of water) by heating the filtrate each time to boiling and pouring it over the precipitate. Mercuric sulphide does not dissolve (A), whereas sulphides of lead, copper, bismuth and cadmium pass into solution as nitrates (B).

A. Examination of Residue upon Filter for Mercury

A residue, remaining after extraction with hot nitric acid of the portion of the hydrogen sulphide precipitate insoluble in ammonium sulphide, should always be tested for mercury, even when it is not pure black or gray-black¹. Treat this residue upon the filter with a little hot, somewhat diluted hydrochloric acid, containing in solution a few crystals of potassium chlorate, and pass the acid through the paper several times. Evaporate the filtrate to dryness in a porcelain dish upon the water-bath, and dissolve the residue in 2-3 cc of water containing hydrochloric acid. Filter this solution and examine the filtrate for mercury:

(a) **Stannous Chloride Test.**—Add a few drops of stannous chloride solution to a portion of the filtrate. If mercury is present, a white precipitate of mercurous chloride will appear. Excess of stannous chloride, especially if heat is applied, will reduce this precipitate to gray, metallic mercury.

(b) **Copper Test.**—Put a few drops of the filtrate upon a small piece of bright copper. Mercury immediately deposits a gray spot having a silvery luster when rubbed. Wash the copper, upon which mercury is deposited, successively in water, alcohol and ether. Dry thoroughly in the air and heat in a small bulb-tube of hard glass. Mercury will sublime and collect in small, metallic globules on the cool sides of the tube. A trace of iodine vapor, introduced into the tube, immediately converts the gray sublimate into scarlet mercuric iodide (HgI_2).

(c) **Phosphorous Acid Test.**—Add some phosphorous acid to another portion of the filtrate and warm gently. If mercury is present, a white precipitate of mercurous chloride¹ will appear.

(d) **Mercuric Iodide Test.**—Add 1-2 drops of very dilute potassium iodide solution to the remainder of the filtrate. A red precipitate (HgI_2), readily soluble in excess of potassium iodide, shows presence of mercury.²

¹ $2\text{HgCl}_2 + \text{H}_2\text{O} + \text{H}_3\text{PO}_3 = 2\text{HgCl} + 2\text{HCl} + \text{H}_3\text{PO}_4$.

² (a) $\text{HgCl}_2 + 2\text{KI} = \text{HgI}_2 + 2\text{KCl}$. (b) $\text{HgI}_2 + 2\text{KI} = \text{K}_2^{++}(\text{HgI}_4)^{-}$.

B. Examination of Nitric Acid Solution

The nitric acid solution may contain nitrates of lead, copper, bismuth and cadmium. Evaporate this solution in a porcelain dish upon the water-bath nearly to dryness and dissolve the residue in a little hot water. Add dilute sulphuric acid to the filtered, clear solution. If lead is present, a heavy white precipitate of lead sulphate, soluble in basic ammonium tartrate, will appear. Test the filtrate from the precipitate of lead sulphate, or the solution remaining clear upon treatment with sulphuric acid, next for copper and bismuth.

(a) **Copper and Bismuth Tests.**—Add ammonia in excess to the greater part of the filtrate. It will produce a blue color, if copper is present. A white precipitate at the same time may be bismuthous hydroxide.¹ For the positive detection of bismuth, wash the precipitate and dissolve upon the filter in a few drops of hot, dilute hydrochloric acid. Pour this solution into considerable water. A white precipitate of bismuthous oxychloride (BiOCl) proves presence of bismuth.

For additional proof of bismuth, redissolve the white precipitate in dilute hydrochloric acid and divide the solution into two parts. Add a few drops of stannous chloride solution to one part and then sodium hydroxide solution until the reaction is strongly alkaline.² This will produce a black precipitate of metallic bismuth. Add a few drops of sulphurous acid and potassium iodide solution to the second part of the hydrochloric acid solution. If bismuth is present, an intense orange-yellow or only a yellowish color will appear. This is a very delicate test, making it possible to detect with certainty 0.01 mg. of bismuth in 1 cc. of solution.

(b) **Potassium Ferrocyanide Test.**—Add potassium ferrocyanide solution to another portion of filtrate from lead sulphate, or solution remaining clear upon treatment with sulphuric acid. According to the quantity of copper present, a brown-red precipitate of copper ferrocyanide ($\text{Cu}_2\text{Fe}(\text{CN})_6$) immediately appears, or only a brown-red coloration yielding flocks of the same color upon standing.

¹ Ammonium hydroxide solution does not precipitate pure bismuthous hydroxide ($\text{Bi}(\text{OH})_3$) from solutions of bismuth salts but a basic salt, the composition of which depends upon temperature and concentration of the solutions.

² If stannous chloride solution produces in acid solution a gray or black precipitate, it is due to mercury; bismuth is reduced only in alkaline solution by the stannous salt, that is, by sodium stannite ($\text{Sn}(\text{ONa})_2$).

(c) **Precipitation of Metallic Copper.**—Put a bright knife-blade, or iron nail, in the solution to be tested for copper. If copper is present, a red film of copper will be deposited upon the blade or nail.

To detect cadmium in presence of copper, add potassium cyanide to the blue solution in (a) produced by excess of ammonia, or to the blue filtrate from bismuthous hydroxide, until the color is discharged and then pass in hydrogen sulphide. Cadmium is precipitated as yellow sulphide (CdS), whereas copper will remain in solution as colorless potassium cuprocyanide ($\text{K}_6\text{Cu}_2(\text{CN})_8$).

When copper is absent, test for cadmium direct by passing hydrogen sulphide into the ammoniacal solution obtained in (a). If a reddish or brownish instead of a yellow precipitate appears, filter and heat the dried precipitate upon charcoal in the blow-pipe flame. Cadmium will give a brown coating.

METALLIC POISONS III

Examination for Chromium and Zinc

Test the filtrate from the hydrogen sulphide precipitate (page 248) for chromium and zinc. Concentrate the filtrate in a porcelain dish to about one-third its original volume and divide this solution into two parts.

To test for zinc, add enough ammonia to render one-half the concentrated filtrate alkaline. This treatment usually gives the solution a dark color. Then add ammonium sulphide solution in excess. This reagent almost always produces a precipitate even when zinc is absent, for solutions prepared from animal and vegetable materials usually contain iron compounds and phosphates of the metals of the earths and alkaline earths. When this precipitate has settled, add acetic acid until the solution has a faint acid reaction. Stir the mixture thoroughly and allow it to stand for some time. The color of the precipitate becomes lighter because acetic acid partially dissolves sulphide of iron. Moreover phosphates are partially dissolved, except ferric phosphate (FePO_4) which is insoluble in acetic acid. Collect the precipitate upon a filter, wash, dry and ignite precipitate and filter in a porcelain crucible. Before ignition, moisten the filter with a concentrated solution of ammonium nitrate to prevent reduction of zinc compounds, when ignited with carbon from the burning filter, to metallic zinc and volatilization of the latter. Extract the residue from ignition with dilute sulphuric acid, filter and divide the filtrate into two parts.

(a) Add sodium hydroxide solution in excess to one portion of the filtrate and shake thoroughly. Filter, to remove the white precipitate of ferric phosphate that almost always appears, add a few drops of ammonium or hydrogen sulphide solution to the clear filtrate and warm gently. Zinc, if present, gives a white, flocculent precipitate of zinc sulphide.

(b) Add ammonia in excess to the second part of the filtrate. Filter, to remove any precipitate that appears, acidify the filtrate with acetic acid, warm and pass in hydrogen sulphide. Zinc, if present, will appear as the white, flocculent sulphide.

(c) As an additional test for zinc, dissolve in a few drops of hot dilute hydrochloric acid the precipitate produced by ammonium or hydrogen sulphide (as described above in *a* and *b*), after it has been collected upon a filter and washed. Boil for some time to expel hydrogen sulphide and filter to remove precipitated sulphur. Add potassium ferrocyanide solution to the clear, cold filtrate. Zinc ferrocyanide ($\text{Zn}_2\text{Fe}(\text{CN})_6$) is precipitated white, slimy and nearly insoluble in dilute hydrochloric acid.¹

To test for chromium² in the second part of the filtrate from the hydrogen sulphide precipitate, concentrate the solution in a porcelain dish to a small volume. Then add twice the quantity of potassium nitrate and sodium carbonate until the reaction is strongly alkaline and heat until perfectly dry. Add this dry residue in small portions to a little potassium nitrate fused in a crucible. In fusing a large quantity of material, it is advisable to use a large, bright nickel crucible which is especially adapted for this operation. When fusion is complete and all organic matter is oxidized, cool, boil crucible and contents in a porcelain dish with not too much water, and filter the solution. If the filtrate contains chromium, it will have a more or less intense yellow color. Even traces of chromium³ may be

¹ Excess of potassium ferrocyanide combines with zinc ferrocyanide first precipitated, forming insoluble potassium zinc ferrocyanide:



² In testing for metallic poisons, chromic oxide (Cr_2O_3), insoluble in acids, may be disregarded as it is not poisonous.

³ Two drops of 10 per cent potassium chromate solution (= 0.01 gram of K_2CrO_4) in 500 cc. of water produce a marked yellow color. Fifty cc of this solution, containing 1 mg of K_2CrO_4 , have a yellow color that can still be recognized with certainty.

recognized by the yellow color of the filtrate. When the solution of the melt is colorless, it is unnecessary to test for chromium. To detect chromium with certainty, when the filtrate is yellow, divide the solution into two portions and make the following tests.

(a) **Chrome Yellow Test.**—Add acetic acid in excess to one portion of the filtrate, boil for some time to expel carbon dioxide and nitrous acid, and add a few drops of lead acetate solution. A yellow precipitate of lead chromate (PbCrO_4) will appear, if chromium is present. When the precipitate is mixed with considerable lead sulphate or chloride, the color is only yellowish. A white precipitate is due to PbSO_4 , PbCl_2 or $\text{Pb}_3(\text{PO}_4)_2$. When the aqueous solution of the melt is colorless, such a precipitate is usually obtained.

If solutions of potassium nitrite, always formed in potassium nitrate fusions, lead acetate and acetic acid are mixed, the resulting solution has a strong yellow color. A white precipitate in such a solution may appear to have a yellow color. To eliminate this source of error, allow the precipitate to settle, collect upon a filter, and wash thoroughly. If it is pure white, chromium is absent.

(b) **Reduction Test.**—Add sulphurous acid to the second portion of the yellow filtrate. The yellow color will change to green, or green-blue, with formation of chrome alum. This is not as delicate as the preceding test.

METALLIC POISONS IV

Examination of Residue from Hydrochloric Acid and Potassium Chlorate for Barium, Lead, and Silver

Wash thoroughly with water the residue, undissolved after treatment with hydrochloric acid and potassium chlorate, and dry in an air-closet or upon a porous plate. Then add three times the quantity of a mixture of 2 parts of potassium nitrate and 1 part of dry sodium carbonate and triturate in a mortar together with the filter. Gradually introduce this mixture into a hot porcelain crucible. In this operation organic substances (fat, fatty acids, etc.) are oxidized by potassium nitrate with considerable deflagration. Finally when all material is in the crucible, add 0.5 gram more of potassium nitrate. Cool the melt, soften with hot water, wash into a flask, and pass carbon dioxide for several minutes through the liquid which is usually turbid. This treatment converts caustic

alkali formed during fusion into carbonate and completely precipitates any lead that may be in solution. Then heat the solution to boiling and allow to settle for some time. Collect upon a small filter the deposit¹ which may contain barium carbonate, basic lead carbonate and metallic silver. The latter will impart a gray color to the deposit. Thoroughly wash the precipitate with hot water and dissolve upon the paper in hot, rather dilute nitric acid,² passing the acid through the paper several times. Evaporate this nitric acid solution to dryness in a porcelain dish. Dissolve the residue in water, heat the solution to boiling, and precipitate silver with dilute hydrochloric acid. Filter, to remove silver chloride, and pass hydrogen sulphide through the filtrate to precipitate lead. To test for barium in the filtrate from lead sulphide, or in the solution that gave no precipitate with hydrogen sulphide, heat to boiling to expel hydrogen sulphide and filter to remove insoluble matter. Then add dilute sulphuric acid which will precipitate barium sulphate. The following tests of identification should be applied to these several precipitates.

For the further identification of silver, dry the precipitate caused by hydrochloric acid and fuse in a porcelain crucible with a little potassium cyanide. Extract the cold melt with hot water. Metallic silver will remain undissolved.

To confirm presence of lead, dissolve the hydrogen sulphide precipitate in hot nitric acid, and evaporate the solution to dryness. Dissolve the residue in water, filter and test the solution for lead with dilute sulphuric acid, potassium chromate and potassium iodide.

As additional proof of presence of barium, collect the sulphuric acid precipitate upon paper, wash thoroughly and test upon a clean platinum wire in a non-luminous flame. Barium imparts a yellowish green color to the flame. To avoid any mistake, examine this flame with the spectroscope. In forensic-chemical examinations these reactions of identification should always be carried out with the utmost care!

¹ Even in absence of barium, lead and silver, such a deposit usually appears. In that case it probably consists of material from the porcelain crucible, the glazing of which is attacked to some extent in the process of fusion.

² Use 5-6 cc. of an acid prepared by mixing 1 volume of concentrated nitric acid with 2 volumes of water.

TOXIC ACTION OF ARSENIC, ANTIMONY AND METALLIC POISONS AND ELIMINATION FROM THE HUMAN ORGANISM

Absorption and Toxic Action of Arsenic

Arsenious and arsenic acid exert a strong corrosive action that manifests itself upon both the outer skin and mucous membranes. Rapid absorption of these arsenic compounds is ascribed to such action. How far-reaching absorption of arsenic through the outer skin is appears from the fact that fatal poisonings have repeatedly followed use of arsenic plaster. As a result of paralyzes after absorption, blood-pressure falls and the abdominal organs are highly congested. This extends especially to the mucous surface of the gastro-intestinal tract, giving it a bright red appearance as in severe gastro-intestinal catarrh. This apparent gastro-enteritis is rendered more intense by the fact that absorbed arsenic is eliminated not only by the kidneys but also by the glands of the intestinal mucous membrane in consequence of which they are very severely irritated. Like phosphorus all arsenicals cause severe disturbances of metabolism, recognized by fatty degeneration of almost all organs of the vascular parenchyma of the kidneys, liver, cardiac muscle, and gastro-intestinal epithelium.

Brain and spinal cord also suffer severe injury. Disorders of the skin frequently appear. Such effects are explained by elimination of small quantities of arsenic in the skin which then give rise to oedema and pigmentation. Ill effects due to arsenic manifest themselves in mucous membranes as angina, hoarseness, cold, persistent bronchial catarrh and inflammation of the eyes. In case death does not occur too soon, arsenic finally is deposited in the bones. According to Kobert, this deposition may be regarded as a kind of natural cure, for all symptoms of poisoning disappear at this stage because arsenic deposited as calcium arsenate, $\text{Ca}_3(\text{AsO}_4)_2$, is redissolved only with difficulty.

According to Brouardel,¹ poisoning by arsenic usually takes place in four phases. In the first phase the gastro-intestinal tract and kidneys are attacked, in the second other abdominal organs, organs of the chest, and central nervous system. In the third phase skin, hair and nails are affected, and in the fourth the poison not eliminated is deposited in the bones as calcium arsenate and there fixed. In very rare cases of poisoning, the first series is said apparently to be entirely omitted so that symptoms of nervous paralysis appear at once. In most cases the first symptoms of poisoning do not appear for a half to a full hour. In the rarer, the paralytic form, uniform paralysis of the central nervous system and heart is the predominant symptom and may result in death within 10 hours. In the gastro-intestinal form, the manifestations of paralysis, as compared with gastro-intestinal symptoms, are very much suppressed. The effects noted are: emesis, great thirst, dryness and itching in mouth and throat, difficulty in swallowing, extremely violent pains in the abdomen, severe, foul-smelling diarrhoea having the appearance of rice-water or dysenteric. As a result secretion of the urine is diminished and it usually contains blood and cylinders and very soon

¹Brouardel. Les intoxications. Paris 1904.

also arsenic and sugar. In the next place the phenomena that usually appear are those of the heart and nervous system, that is, headache, dizziness, pain in the limbs, low blood-pressure, weak irregular pulse, coldness of extremities, syncope, coma, convulsions, and general paralysis. In many cases disorders of the skin appear, having the character of an eczema or giving the picture of erysipelas with formation of little blisters, usually on the face and genitals. The contents of these blisters are said to contain arsenic. Even in milder skin affections general scaling of the skin together with loss of hair may appear. In addition, cases are described in the literature where only gastro-intestinal phenomena have been observed. Frequently this gastro-enteritis results in fatal collapse within 18-70 hours. In rarer cases death ensues more rapidly, within 10 hours, as in the paralytic form of arsenical poisoning. In not a few instances death comes on much more slowly, that is, after 4-14 days.

Lethal Dose.—According to Kobert, even 0.1-0.3 gram of white arsenic (As_2O_3) may produce fatal results. Arsenates are less toxic than arsenites. Elimination of arsenic in urine begins 6-12 hours after the arsenical preparation has been taken and lasts as a rule after a single dose 4-10 days. Upon the basis of many experiments, different authors have set the duration of arsenic elimination at a much longer time, for they have succeeded in detecting it in urine 80 and even 90 days after poisoning. Therefore in suspected arsenic poisoning the urine of the patient first of all should be tested for this poison. In arsenic poisoning the quantity of urine is usually much diminished and almost always contains albumin and blood-corpuscles.

Distribution and Deposition of Arsenic in the Human Body

In a series of investigations published in 1880 Ludwig¹ furnished positive proof that the liver is the principal depository of arsenic administered by the mouth. Ludwig examined different parts removed at post-mortems from cadavers of persons who had died of arsenical poisoning. He also determined the quantity of arsenic in different parts of cadavers of dogs in which acute and chronic arsenical poisoning had been produced. Ludwig undertook this investigation mainly because of a research carried out in 1876 in Gautier's laboratory by Scolobusoff. The latter had reached the conclusion that in acute poisoning arsenic first reaches the brain and then quickly passes into the spinal cord, and also that in chronic poisoning most of the arsenic remains in the brain and spinal cord; whereas the liver and muscles contain much smaller quantities of arsenic. In one case Scolobusoff found 40 times more arsenic in the brain and spinal cord than in the same weight of muscle and 3 times more than in the same weight of liver. On the contrary, according to Ludwig, both in acute and chronic arsenical poisoning only extremely small quantities of arsenic are found in the brain, whereas a large quantity of arsenic can be detected both in the liver and kidneys, particularly in the latter organ in acute cases. In chronic poisoning, if administration of the poison is stopped, arsenic is retained longest in the liver. Ludwig found arsenic in abundance in the liver of a dog 40 days after the last dose, whereas the brain, bones and muscle contained no arsenic.

¹ E. Ludwig: Distribution of Arsenic in the Animal Organism after Administration of Arsenious Acid. *Med. Jahrb. Wien.* 1880, 4H

Contrary to Armand Gautier, who claims to have found arsenic occurring normally in the human thyroid gland, Hödlmoser¹ found this organ as well as the liver of individuals, who had died from various diseases, free from arsenic without exception. Hödlmoser also obtained entirely negative results from experiments with thyroid glands of sheep and swine, and from the nuclein prepared from these by peptic digestion. Arsenic could not once be found in minute traces in the thyroid glands examined.

In a forensic-chemical examination the contents of the stomach and intestines should claim attention first of all, for sometimes poison that has not been absorbed may still be found there. Among the organs taking up arsenic, the liver should be considered first, for in arsenical poisoning usually the poison is deposited there in largest quantity. Other organs that should be examined are the spleen, heart, kidneys and lungs. In most instances the brain contains merely traces of arsenic, contrary to the claim of Scolobusoff and Gautier that in both acute and chronic poisoning the brain and spinal cord contain the largest deposits of arsenic. The ectodermal organs also appear to be concerned in the fixation and elimination of arsenic, for this poison may accumulate in hair, skin and nails. In 4 cases of severe salvarsan poisoning with fatal termination, the author determined the quantity of arsenic in organs and urine of these individuals, men varying in age from 30-38 years, and in every instance found most of the arsenic in the liver and only traces in the kidneys and urine. In 3 cases the brain was found arsenic-free, although in each only half of the organ was examined, in the brain of the fourth individual minute quantities of arsenic were detected.

Johnson and Chittenden² also found in arsenical poisonings most of the poison in the liver. In the case of a woman poisoned by arsenic trioxide they found the following quantities of metallic arsenic:

Organ	Metallic Arsenic
Stomach and spleen	0.0104 per cent.
Kidneys	0.0083 per cent.
Liver	0.0081 per cent.
Lungs and heart	0.0033 per cent.
Uterus and intestines	0.0026 per cent.
Lungs and fluid from the thorax	0.0014 per cent.
Bladder	noticeable traces
Brain	unweighable traces

The quantity of arsenic was also determined in bones and muscles. It varied from 0.00016-0.00086 per cent. in the individual parts of the arm and leg.

Similar results were obtained from the cadaver of a dog fed for 8 days with arsenic and examined 24 hours after the last, non-lethal dose. Intestines 0.002; liver 0.001, kidneys 0.0005, muscles 0.0002; urine taken from the bladder 0.0002 per cent. of arsenic. The blood contained noticeable traces of arsenic, whereas the brain was nearly arsenic-free.

¹ C. Hödlmoser. Do Certain Organs of the Human Body Contain Arsenic Physiologically? *Zeitschr. f. physiol. Chem.* 33 (1901), 328.

² S. W. Johnson and R. H. Chittenden. Distribution of Arsenic in the Human Body in a Case of Arsenic Poisoning. *Amer. Chem. Journ.* 2 (1881), 332.

Recently Gascard¹ published two cases of arsenical poisoning, one running a slow and the other a rapid course. In one case (I) a woman died 22 hours after eating a cake containing about 1 gram of arsenic trioxide. Arsenic was determined in organs that had not begun to putrefy. In the other case (II) a man took about 0.4 gram of arsenic in soup. Although he received immediate medical attention and had his stomach washed out, death ensued after an illness of 9 days. The following quantities of arsenic, calculated in each case for 1 kilogram of organ, were found:

Organ	Case I	Case II
Stomach	124 mg. As_2O_3	2 mg As_2O_3
Small intestine	223 mg As_2O_3	6 mg As_2O_3
Liver	44 mg As_2O_3	8 mg As_2O_3
Kidneys	14 mg As_2O_3	7 mg As_2O_3
Brain	0.1 mg As_2O_3	5 mg As_2O_3

A noticeable fact about Case II is that the quantity of arsenic in the brain is about 50 times more than in Case I. This may be characteristic of arsenical poisoning running a slow course and is probably closely related to disturbances of the nervous system, frequently observed in such poisonings.

Ekeley² determined the quantity of arsenic in organs and other parts of the body of a man who had died from arsenical poisoning. The cadaver had lain in the ground 6 weeks before the examinations were made. The largest quantities of arsenic were found in the stomach, liver and kidneys. On the other hand, in percentages the kidneys and walls of the stomach contained most arsenic, next the liver and walls of the intestines, the heart somewhat less, and the brain and spinal cord only a trace. Muscular tissue, especially the thigh and toes contained quite considerable quantities of arsenic. Since arsenic was also found in these, Ekeley assumes that muscular tissues carry on distribution of arsenic through the body.

Heffter³ has made the following statements with regard to deposition of arsenic in hair. When inorganic or organic arsenical compounds have been taken once or several times, arsenic is stored in the hair. Arsenic enters only into living hair. Dead hair on the contrary will remove nothing from an arsenical solution. Arsenic is firmly held in hair in a form of combination insoluble in water and alcohol. In such cases the amount of arsenic in hair varies from 1 to 5·100,000. In point of time, passage of arsenic into hair takes place later than deposition of arsenic in abdominal organs. Consequently in acute poisonings running a rapid course arsenic is found in abdominal organs but not in hair. After chronic administration of arsenical preparations medicinally, arsenic has been found in hair at least 14 days after medication began. Disappearance of arsenic from hair requires a very long time so that it may be detected years after, even when

¹ A. Gascard: Two Cases of Poisoning by Arsenic Trioxide, one Acute and the other prolonged. *Journ. Pharm. Chim.* (7) 7 (1913), 329.

² J. B. Ekeley: Distribution in the Human Body of Arsenic administered before Death. *Journ. Amer. Chem. Soc.* 35 (1913), 483.

³ A. Heffter: Deposition of Arsenic in Hair. *Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw.* (3) 49 (1915), 194.

arsenic has long since left the liver and bones. Detection of arsenic in liver and kidneys and its absence at the same time in hair indicate acute arsenical poisoning. If, however, arsenic is found in hair and other parts of the body contain none, the supposition is in some cases that the poison was taken many years ago. On account of the usually high arsenic-content of hair, 5 grams are sufficient for an examination.

Normal Occurrence of Arsenic (?), Copper and Zinc in the Human Organism

In case of many metals introduced into the human body, the liver is the most important organ concerned in taking them up and frequently contains small quantities of heavy metals that have usually found their way into the body with food. Copper, at least in traces, is frequently encountered in the liver during toxicological examinations. Lehmann¹ has given the following figures for the quantity of copper in 1 kilogram of liver. Man 2.5-5 mg, beef-cattle 22-51 mg; calf up to 48 mg, sheep up to 18 mg.

Van Itallie and van Eck² found that 5 mg of copper in 1 kilogram of liver, the maximum quantity given by Lehmann, was almost uniformly exceeded in the case of Dutch livers. They found 3.2-19 mg of copper in the same quantity of liver. Copper is apparently eliminated in the liver even during foetal life and in larger quantity than in succeeding periods of life. At least these investigators found 26.1 mg of copper in the liver of a still-born child and 30 mg in that of a child several hours old, referred to 1 kilogram of the organ.

According to van Itallie and van Eck (loc cit), zinc is said to occur almost uniformly in the human liver. They found quite considerable quantities, that is, 17-87 mg of zinc in 1 kilogram of liver. In examining livers for metallic poisons, the author has repeatedly found traces of copper but zinc only very rarely. In case such traces of copper or zinc are found in the liver or in any other organ, the greatest care must be observed in drawing conclusions from the results. Without further investigation in a case of this kind the conclusion should not be drawn that poisoning from one or the other metal has taken place. Raoult and Breton³ also determined copper and zinc in human livers and found quantities, varying between 3-15 mg of copper and 10-76 mg. of zinc in 1 kilogram of the organ. Ogier⁴ before that had observed "On trouve du zinc dans les organes souvent, mais pas toujours, de même qu'on y trouve parfois du cuivre. Mais il s'agit toujours de très petites doses."⁵

According to van Itallie and van Eck, on the other hand arsenic, contrary to the statement of Gautier, is not a normal constituent of human liver. Gautier⁶

¹ Lehmann Occurrence and Estimation of Copper in Organic Substances Arch. f. Hyg. 24, 1. 18 (1895), 73

² L. van Itallie and J. J. van Eck. Occurrence of Metals in the Liver. Pharm. Weekblad 49, 1157, Arch. d. Pharm. 251 (1913), 50

³ Raoult and Breton. Natural Occurrence of Copper and Zinc in the Human Body. Compt. rend. de l'Acad. des Sciences 85 (1877), 40.

⁴ Traité de chimie toxicologique, page 351.

⁵ "Zinc is often found in organs but not always, just as copper is sometimes found. But it is always a matter of very small quantities."

⁶ A. Gautier. Report concerning the International Congress of Pharmacy in Paris in 1900; also Pharmazeutische Zentralhalle 41, 502

claims to have found traces of arsenic in the thymus gland and brain of all carnivorous and herbivorous animals as well as of man, and even 0.001 gram in 0.7 gram of thyroid gland. Moreover Schlagdenhauffen and Pagel found arsenic in the thymus glands of various young animals and in the testicles and ovaries of man, oxen, cows and horses. Van Itallie and van Eck examined the livers of 25 persons of very different age and different sex for copper and zinc and found the values given above for these metals. They were able only once to detect arsenic positively in a human liver. The deceased, however, previous to death had taken arsenic medicinally! Obviously in forensic-chemical investigations the almost normal occurrence of copper and zinc in traces in the liver must be taken into consideration. These two investigators were unable to find any relation between sex, age, occupation, residence and the quantities of copper and zinc observed.

ANTIMONY

Experiments upon animals, into the blood of which, or subcutaneously, sodium tartar emetic was injected, have shown that the action of antimony is very similar to that of arsenic, the only difference being that poisoning appears more slowly. On the other hand, the action of tartar emetic taken by the mouth is not identical with that of arsenic. It differs from arsenic in being a stronger local irritant. Owing to the strong local irritation of the stomach, even doses that are not lethal produce emesis through reflex action, whereas arsenic produces emesis only in toxic doses and for this reason cannot be employed therapeutically as an emetic. Moreover it is possible to produce pustulous skin eruptions more surely with tartar emetic ointments than with those containing arsenic.

After internal administration, antimony is eliminated in great part before absorption by emesis. A part of the poison may be deposited in an insoluble condition in the liver and possibly also in the bones. Even traces of antimony have also been found in the brain and spinal cord. Pouchet found antimony in the skin, hair, bones and especially in the intestinal tract. Materials to be taken into consideration for chemical examination are vomitus, mucous membrane of stomach and intestines, stomach and intestinal contents, liver, and also brain, skin, hair and urine.

BARIUM

Of the barium compounds, only barium hydroxide, sulphide and more concentrated solutions of neutral barium salts act as local irritants. On the other hand, all soluble barium salts may produce effects due to absorption. These concern the heart and blood-vessels, gastro-intestinal canal, central nervous system, that is, picrotoxin-like stimulation then paralysis, and muscles. Elimination of barium takes place by way of the salivary glands, intestinal glands, and in small quantity by the urine.

The effects of intoxication are nausea, flow of saliva, retching, emesis, very painful colic, and severe diarrhoea. A noticeable feature is the digitalis-like influence upon the heart and blood-vessels, manifesting itself in slow pulse and strong palpitation of the heart. The blood-pressure is strikingly increased. Additional symptoms are dizziness, ringing in the ears, deafness, visual disturb-

ances and a feeling of anxiety. Catarrhal affections of the conjunctiva and of the mucous membrane of the respiratory tract, that is, of the nose, have been observed in man (R Kobert). In one case described in the literature, 10 grams of barium chloride proved to be a lethal dose, in another case 3 grams of the same barium salt proved fatal. The autopsy shows in the mouth swelling of the salivary glands, the stomach and duodenum in particular exhibit inflammatory changes and are very red, the heart, lungs and brain are highly congested.

Experiments upon animals have shown that distribution of barium in fatal poisoning is as follows: the lungs, heart and muscles contain only traces, the liver, kidneys, brain and spinal cord a little more, and the bones most of the barium. Only a small part of the barium is eliminated in the urine. The parts of the cadaver of most importance for chemical examination are vomitus, stomach-contents, faeces and urine, the latter especially after rather large doses of barium have been taken.

TOXIC ACTION OF HEAVY METALS

Most salts of heavy metals, such as lead, copper, mercury, silver, uranium, bismuth and zinc, produce precipitates with protein substances, forming metal-albuminates. In combining with oxides of these metals, proteins behave like acids. If the metal-albuminates first formed are insoluble, or only slightly soluble in the body-fluids, they are non-toxic or only slightly toxic. But a soluble albuminate is transported throughout the organism and exerts a toxic action. Every cell in contact with the dissolved metal may be poisoned. Mercury albuminate is an example of the latter class of metal-albuminates. On account of its solubility in sodium chloride and protein solutions, it acts as a powerful poison. Copper albuminate on the other hand is not appreciably soluble in solutions of sodium chloride, hydrochloric acid or proteins. Not entering the circulation, it is as good as non-toxic. Lead and silver albuminates are like copper albuminate as regards solubility in the solvents mentioned. But if a heavy metal, forming a difficultly soluble albuminate, finds its way into the organism in organic combination, that is, in a non-ionizable form, for example, copper in union with tartaric acid, so that it cannot be precipitated by proteins, then it is as poisonous, or nearly as poisonous, as mercury in corrosive sublimate. Administration intravenously of 20 mg of such copper causes the death of an adult rabbit.

Consequently precipitation takes place wherever the salt of a heavy metal comes in contact with proteins. The term corrosion is applied to such an occurrence. There is always present the metallic oxide, protein and acid originally combined with the metal. As a rule the acid is loosely held by the precipitate and is washed away by the circulating blood. The corrosive action of salts of heavy metals is due both to the union of metallic oxide with protein, living protein being changed to dead metal-albuminate, and to the caustic action of free acid. Therefore the intensity of the action of the salt of the heavy metal depends upon the nature of the metal-albuminate. The degree of solubility in the body fluids is especially important, as well as the quantity and strength of free acid. Salts of heavy metals not only may affect the place of application, but they may give rise to serious changes where eliminated, as in intestines or

kidneys Before elimination they may also seriously harm parenchymatous organs like the liver, as well as circulatory organs Finally salts of heavy metals have an important action upon the blood R Kobert and collaborators have found that white as well as red blood-corpuscles may combine with metals and act as antidotes Kobert has shown that the substance of red blood-corpuscles is capable of taking up a considerable quantity of a heavy metal A chemical compound (metal-haemoglobin) is formed and the oxyhaemoglobin spectrum is not changed Thus lead speedily impairs vitality of red blood-corpuscles Consequently red blood-corpuscles are killed in large quantity in lead poisoning

LEAD

Lead is eliminated in urine and faeces Elimination by faeces always exceeds that by urine, even when lead has not been taken by the mouth Mann,¹ for example, in the case of two patients was never able to find more than 0.6 mg of lead in urine collected during 24 hours, whereas faeces during the same period contained 2-3 mgs of lead In lead poisoning the metal has been found in saliva, bile and blood, both in red and white blood-corpuscles In animals relatively most of the lead has been found in the kidneys, after which come bones, liver, testes and finally brain and blood In experiments with sheep, Ulenberger and Hofmeister obtained the following results. Kidneys 0.44-0.47, liver 0.3-0.6, pancreas 0.54, salivary glands 0.42; bile 0.11-0.40; bones 0.32, faeces 0.22, spleen 0.14, blood 0.05-0.12, urine 0.06-0.08 gram per 1000 grams of each material examined

Lead is eliminated especially by the bile and in acute poisoning this secretion may contain more lead than any of the other organs or secretions Oliver² obtained the following results from human material. Liver 41.6, spleen 39.0, large brain 21.6, small brain 8.6, kidneys 10.0, and heart 0.5 mg per 1000 grams of each material examined Elimination of lead by the urine in the case of man is not always uniform Urine is free from lead for a long time and later without further administration again contains the metal

Elimination of lead after administration proceeds very slowly by the urine, intestinal glands, skin, saliva and possibly the milk During continuous administration of small doses of lead salts, therefore, cumulative action takes place Brouardel considers even a daily dose of only 1 mg. of lead sufficient to produce chronic plumbism in man If antidotes are not used, the poison in part may be retained in the organism for many months The metal is abundantly eliminated through the kidneys only while the body is overloaded with lead Elimination proceeds later chiefly through the intestines But elimination may cease almost entirely for months notwithstanding the severest symptoms of intoxication. Of all metals none causes such severe injury to the kidneys as lead, for it is capable of producing serious impairment of this organ Even small quantities of lead in passing through the kidneys bring about lasting illness in the secreting parenchyma of this organ, laying it waste and ultimately causing true cirrhosis of the kidneys (Kobert) In chronic lead poisoning in rabbits Oppen-

¹ Zeitschr. f. physiol. Chemie 6 (1882), 6.

² The Lancet, March 1891.

heimer found the following quantities: Brain 199.7, hollow bones 118, kidneys 62.7; bone-marrow 57.7, liver 48, muscles 33.3, and blood 4.9 mg of lead per 100 grams of each dry material examined. Therefore the brain sometimes may contain quite an abundance of lead! A human brain was also found to contain over 100 mg of lead in 100 grams of dry material. Blyth isolated relatively more lead from the small brain than from the large brain of a man who had died from *Epilepsia saturnina*, obtaining 117 mg of lead sulphate from the entire organ. In another case Blyth obtained 106 mg of lead sulphate from the entire brain. In one instance the lungs were also found to contain quite a quantity of lead. As a rule lead is so firmly deposited in these organs that the metal can be detected years after in persons formerly poisoned with lead. Lead can also be detected for a long time in the cadaver. In the examination of an exhumed cadaver special consideration should be given to the fact that lead may reach the cadaver later from ornaments or pigments of the coffin containing this metal. Moreover it should be borne in mind that lead is deposited in the brain, spinal cord, bones, bone-marrow, liver, kidneys, intestines and muscles. Consequently in such examinations these parts of the cadaver should first of all be tested for lead.

CHROMIUM

Chromic acid and all soluble chromates and dichromates act as strong poisons. Alkaline chromates are absorbed by the mucous membranes, producing severe, acute poisoning. The poison causes intense pain in the stomach and intestines, collapse and kidney derangement that may terminate fatally in a few hours. Other symptoms are nausea, vomiting of yellow matter that is later tinged with blood, diarrhoea and even bloody stools, intense thirst, emaciation, great anxiety, severe pain in the abdomen, small, feeble and quickened pulse—"the cholera picture" (Kunkel, *Toxikologie*). Statements with regard to the quantity of an alkaline chromate, capable of producing acute poisoning, agree fairly well. Even a few decigrams (0.2 gram) may cause sometimes dangerous symptoms. Chromic acid is eliminated mainly by the urine and partly by the intestines. Elimination takes place rapidly and the body is soon free of the poison. Four days after administration of quite large quantities of a chromate, the urine and faeces are said to contain only traces of this metal. Compounds containing chromium as a positive ion, that is, chromic salts, are about 100 times less toxic than the chromates.

COPPER

Only a small amount of copper, varying with different compounds, is absorbed by the intestines and carried into circulation. Sodium cupric tartrate and copper salts of fatty acids are absorbed most easily. Copper poisoning rarely occurs from introducing a copper compound into the stomach. Copper compounds in large amounts act locally as corrosives, causing severe gastric pains. Vomiting and sense of taste make it impossible to take much of a copper compound. Foods containing copper are unpalatable. Sense of taste as well as after-taste prevent one from swallowing such food in any quantity. Food containing 0.5 gram of copper per kilogram has a marked taste. Irresistible nausea, steadily increasing,

soon makes it impossible to take more of the food containing copper. Elimination of copper by the urine is very slight. Copper absorbed from the intestines is arrested by the liver where it accumulates. Traces of copper have frequently been found in the human liver. In toxicological examinations the author has repeatedly found weighable quantities of copper in livers of adults who had not taken copper salts beforehand, except possibly for suicidal purposes. The liver is the most important organ for detection of copper, next to which come bile, kidneys and gastro-intestinal mucosa. Copper is said to be in the liver as a nuclein compound. In the case of blood, copper is located not in the serum but in the corpuscles.

MERCURY

Mercury is easily absorbed from all mucous membranes, wounds and, in the case of a corrosive mercury compound, such as corrosive sublimate, also from the intact skin. Corrosive action is partly due to precipitation of protein and partly also to the fact that a mercury salt as a result of hydrolysis gives off acid which then exerts corrosive action. Mercury albuminate formed (see *Toxic Action of Heavy Metals*, page 270) enters the circulation, for it is dissolved by alkaline chlorides in the blood. Once in the circulation, mercury is distributed in the kidneys, liver, spleen, intestines, heart, muscles, lungs, brain salivary glands, bile and bones.

Distribution of mercury in the body is said always to be the same, no matter what be the method of administration. It is immaterial whether it is introduced by the mouth, hypodermically, or from an abrasion. Elimination of mercury takes place through the saliva, sweat, bile, gastro-intestinal mucosa, milk and especially through the urine. Elimination in the saliva seems to be constant, since mercury can always be detected in saliva during use of mercurials in lues. A relatively large quantity of this metal is said to be eliminated in sweat. Opinions differ as to the relative quantities eliminated by urine and intestines. Usually elimination by the intestines exceeds that by the kidneys. Recent experiments seem to show that mercury is eliminated in urine regularly and in slowly increasing quantity and slowly diminishes. Elimination of mercury ceases after 6-9 months and even later. In a most favorable case the total quantity of mercury eliminated in urine amounts to about 50 per cent of the total quantity taken but frequently is much less. In mercurial poisoning the kidneys of all organs always contain most mercury and even for weeks. Then follow liver, spleen, bile and intestinal mucosa. In toxicological examinations urine should also be examined, although in acute poisoning it always contains only a fraction of a milligram of mercury in a liter. In severe mercurial poisoning the metal may be said to occur in all organs and secretions.

Soon after administration the action of mercury is apparent. Even after half an hour death may occur or not until days or weeks have passed. Mercury circulating in the blood as mercury albuminate produces inflammation of the mucous lining of the mouth (stomatitis), flow of saliva and severe irritation of the intestines. The latter may give rise to ulcers in the entire colon even when not a trace of mercury has been introduced primarily into the gastro-intestinal tract. Mercurials have a very decided action upon the mouth. It is immaterial whether the preparations are rubbed in, injected, or administered by the mouth. The effects

are flow of saliva, blackening, swelling, and formation of ulcers on gums and tongue. Probably this mercurial stomatitis is connected with elimination of mercury through the saliva and mucous lining of the mouth. Poor teeth actually favor it. Blackening at the margins of the gums is due to formation of mercury sulphide. In severe cases necrosis of the jaws may occur. In short, acute mercurial poisoning manifests itself in inflammation and swelling of the mouth, pains along the digestive tract, vomiting of bloody, mucous masses, bloody stools, and frequently complete anuria. The findings at autopsy are loosened and discolored gums, ulcers, severe inflammation of stomach and intestinal tract, and possibly also perforation. Frequently a slate-gray coloration of the entire mouth, oesophagus, and gastric mucosa is observed. There may also be bleeding and ulcers in the gastric mucosa. Not infrequently acute inflammation and fatty degeneration or calcification of the epithelium of the kidneys are found.

SILVER

In acute poisoning in man the local, corrosive action of silver nitrate in the form of lunar caustic is almost all that has to be considered. With the tissues of the body it forms scabs of silver albuminate that are dry, white and do not extend very deep. These corrosions of the outer skin, resulting from reduction, immediately turn black, especially in the light. All human tissues possess reducing properties but in different degree. The action of living protoplasm in reducing silver solutions is stronger than that of decaying or dead protoplasm.

Urine in acute silver poisoning usually does not contain this metal, whereas it may be found in the intestinal contents even after subcutaneous injection of silver. Part of the soluble silver preparation injected may be seen under the microscope within a few days as brown or deep black points in various organs, such as liver, spleen, kidneys, pancreas, bone-marrow and intestinal mucosa (acute argyria). In chronic argyria the finger nails are first colored dark and frequently also the margin of the gums. This recalls the appearance of the gums in lead and mercurial poisoning but the color is violet, whereas in the case of lead it is more grayish. Later grayish black spots develop also in other parts of the skin and these finally may produce a uniformly dark coloration of the skin where it is exposed to light. Silver salts form such exceedingly stable compounds with protein that there is no way of restoring the natural appearance of skin once discolored by silver. The affinity of protein for silver probably exceeds that of the halogens for this metal. Only a trace of silver reaching the intestines is absorbed. After silver medication, stools are black from silver sulphide. Silver has frequently been determined in organs of persons who have had argyria. In one case the liver contained 0.047 and the kidneys 0.061 per cent. of silver, calculated for the dry organs, and in a second case the liver and kidneys in fresh condition contained respectively 0.018 and 0.03 per cent. of silver.

From every indication the lethal dose of silver preparations is quite high, at least a suicide recovered after having taken 30 grams of silver nitrate.

After administration of silver nitrate there appears as with all corrosives, provided the poison is not immediately expelled by emesis, gastro-intestinal catarrh with burning, severe pain in the abdomen, and vomiting of white masses that turn dark in light. The mucous lining of the mouth is whitish. Dizziness, unconsciousness, paresis, and complete paralysis of individual members of the

body may occur. Death may result from asphyxiating convulsions or from oedema of the lungs (Kobert).

In the cases of poisoning recorded in the literature the autopsy revealed white coloration and superficial scabbing of the mucous membrane of the throat, inflammation and corrosion of the gastro-intestinal tract which here and there was whitish. Dark coloration of the internal organs as well as of the skin was lacking.

URANIUM

Experiments by R. Kobert have shown that uranium, administered subcutaneously or intravenously, is the most toxic of all metals. Uranyl acetate is an excellent precipitant of proteins and other uranyl salts are probably hardly less inferior in this respect. Consequently internal administration of concentrated solutions of uranyl salts destroys the mucous surfaces they touch, for example, that of the stomach, changing the living stomach-wall into dead uranyl-albuminate. Therefore uranyl salts must be classed among the powerful corrosive poisons. In addition to acting as local corrosives, uranium salts resemble hydrocyanic acid in partially arresting internal oxidation in organs and occasioning severest disturbances of metabolism.

Uranium trioxide (UO_3), administered subcutaneously even as a non-corrosive double salt, acts fatally upon cats, dogs and rabbits in doses of 0.5–2 mg. per kilogram of body weight. A very dilute, non-corrosive double salt administered internally in small doses is for the most part unabsorbed. Yet it produces very severe poisoning. Uranyl acetate taken internally produces inflammation of the gastro-intestinal tract. Uranium salts in minimal quantities, such as do not produce gross anatomical changes, give rise to glycosuria and larger doses to albuminuria, cylinduria and blood in the urine. Gastro-intestinal inflammation also appears after subcutaneous administration of non-corrosive sodium uranyl tartrate which is evidence that the poison is eliminated by the gastro-intestinal mucosa. Consequently only traces of uranium are found in the urine. In the chemical detection of uranium the contents of stomach and intestines should receive first consideration.

BISMUTH

This metal becomes quite toxic when it reaches the blood. Bismuth solutions, prepared by dissolving bismuthous hydroxide in tartaric or citric acid and then neutralizing with sodium or ammonium hydroxide solution, have been repeatedly administered to animals subcutaneously and intravenously. The smallest lethal dose of these double bismuth salts, injected subcutaneously, was found to be but 6 mg. per kilogram of body weight for a dog or cat and 24 mg. for a rabbit. Bismuth salts insoluble in water produce entirely different results when administered internally. Bismuth subnitrate and similar salts dissolve very slightly in the highly diluted hydrochloric acid of the gastric juice and consequently very little bismuth is conveyed to the blood. Most of the bismuth taken by the mouth reaches the intestines where it is not absorbed but converted into bismuthous sulphide by hydrogen sulphide always present there. Absorbed bismuth is eliminated by the saliva, bile, urine, mucous lining of the mouth, stomach, small and

large intestine and also milk. If an animal is poisoned by bismuth, the metal can be detected in urine, bile, liver, kidneys, spleen, walls of the intestines, as well as in the bones. Different observers have found the metal in especially large quantity in milk but very little in the kidneys and liver.

Subnitrate of bismuth has given rise to poisonings but is less likely to produce such a result after internal than after external use. Application of 10 per cent bismuth ointment for a burn proved fatal. The gums had a blue-black color and the parenchyma of the kidneys degenerated. Changes in the mouth are similar to those appearing in mercurial poisoning: flow of saliva, swelling of the gums, tongue, salivary glands, and mucous membrane of the cheeks and throat, loosening of the teeth, blackening of the margins of the gums, and ulceration of the mouth. After a cure has been effected, the mucous surfaces attacked remain blackened. Moreover there is intestinal catarrh and nephritis like that caused by corrosive sublimate.

ZINC

Without question zinc salts upon reaching the intestinal tract are absorbed only in small quantities. As yet there is no satisfactory explanation of the fate of absorbed zinc. In zinc poisonings rather large quantities of this metal have repeatedly been found in the liver and bile. This may mean that zinc is arrested by the liver and eliminated in the bile. In an experiment made by Lehmann,¹ a dog was fed for some time with zinc carbonate and killed after 335 days. The following organs, arranged according to the quantity of metal in each, contained zinc: liver, bile, large intestine, thyroid gland, spleen, pancreas, urine, kidneys, bladder, muscle, brain, lymphatic glands, stomach, small intestine, lungs and blood. A fact deserving mention is that quite appreciable quantities of zinc may be acquired from foods. All acids dissolve metallic zinc very freely. Even water containing carbon dioxide is a solvent. Consequently it may be in drinking water from galvanized pipes. All kinds of food and drink, kept in zinc vessels or those coated with zinc, may contain this metal. Moreover plants grown upon soil containing zinc take up this metal. Zinc has also been repeatedly found in parts of human cadavers under circumstances precluding all possibility of poisoning by this metal. Even considerable quantities of zinc have been found in the human liver.

Zinc chloride and sulphate belong to the corrosive poisons, the former in particular having very strong corrosive properties and the latter acting as a powerful emetic. As a result of formation of zinc albuminates, these local corrosive effects produce a firm dry scab. A distinction should be drawn between these corrosive effects and general effects that may manifest themselves in paralysis of the central nervous system soon followed by paralysis of the vascular system, heart and muscles, as demonstrated by experiments where zinc compounds have been injected direct into the blood. Zinc resembles almost all heavy metals in combining with the haemoglobin of the blood. Not only is zinc eliminated by urine but it is said also to be found to some extent in bile and milk. The smallest lethal dose observed after internal administration amounted to 6 grams of zinc chloride and 7.6 grams of zinc sulphate. Occasionally even larger quantities of these two zinc salts have been tolerated.

¹Archiv f. Hygiene 28 (1896), 219.

Poisoning from zinc sulphate resembles acute poisoning from tartar emetic, for it soon produces severe emesis. It differs, however, from the latter in creating an intensely astringent, metallic taste and in giving the mucous lining of the mouth a white, wrinkled appearance. The stomach and intestines may be highly inflamed. Notwithstanding the fact that the stomach was washed out, the tendency to emesis persisted in several poisonings from 50 per cent zinc chloride solution that terminated fatally. Very severe vomiting, often of blood, occurred. In a case observed by v Jaksch, where a drinking glass full of 68 per cent zinc chloride solution containing hydrochloric acid was taken, severe nephritis, fever, and death on the sixteenth day from septic infection ensued. In earlier stages haematoporphyrinuria and glycosuria were in evidence.

TIN

Cases of tin poisoning thus far observed resemble those of copper and zinc. What knowledge there is with regard to the toxic action of absorbed tin has been gained from experiments upon animals. These experiments show that small quantities of tin are absorbed and eliminated in the urine, when ordinary tin compounds are brought into the stomach. But thus far distinct symptoms of poisoning by such quantities of the metal have not been confirmed (Kunkel, *Toxikologie*).

White¹ failed to produce poisoning by introducing a tin compound into a dog's stomach. The animal received sodium stannous tartrate in increasing doses for 22 days, the daily amount being 0.02–0.06 gram. Yet the animal absorbed tin. In the urine, during an experiment lasting 8 days, White found 0.02 gram of tin. But the tin salt mentioned, introduced directly into the circulation of the animal, was quite toxic in action. Stannous chloride, administered for a very long time to a dog, produced symptoms of poisoning. The urine in this case contained small quantities of tin. Kunkel (*Toxikologie*) states that tin has a very slight poisonous action. Apparently it is eliminated very rapidly by the kidneys. Quite probably this prevents accumulation of the metal in the body and consequent poisoning. The fact that White did not observe toxic symptoms, after feeding a dog for 22 days with relatively large quantities of easily absorbable sodium stannous tartrate, and that Ungar and Bodländer² failed to produce derangements with the same compound, until it had been administered for a year, prove that tin is quite free from toxic properties. Hence tin vessels may be used and preserved articles of food containing tin have practically no deleterious action upon health.

Lehmann has come to the conclusion from his experiments that rather large quantities of tin salts may be taken without occasioning more than a milder form of digestive disturbance. Yet these same quantities taken more frequently may also cause disturbance of the nervous system. Quantities of tin, however, necessary to produce the latter effect are far greater than those found in preserves. Consequently ordinary preserves that are not acid, or not strongly so, may be regarded as harmless as far as the quantity of tin they contain is concerned. Lehmann therefore considers it desirable not to put up strongly acid

¹ Archiv f. experimentelle Pathologie u. Pharmakologie 13, 53.

² Zeitschrift f. Hygiene 2, 241.

products, particularly those containing tartaric or malic acid, in tin boxes but in glass, porcelain or wooden containers

SYNOPSIS OF GROUP I

Systematic Procedure in Chemical Examination for Poisons

Before distillation, test a rather small portion of material for phosphorus by **Scherer's test** and a second portion for hydrocyanic acid by **Schönbein's test**.

If this preliminary test for phosphorus is positive, distil in the **Mitscherlich apparatus**, otherwise conduct distillation with Liebig condenser in the usual position. In both cases material should be comminuted as finely as possible, stirred with water to a thin mass, acidified with tartaric acid, and distilled in a capacious glass flask either over free flame or from oil-bath. In many cases it is advisable during distillation to pass through the maternal carbon dioxide washed with water and to warm only upon the water-bath. This is the method used for detection and quantitative determination of hydrocyanic acid and phosphorus in cadaveric material. It is an advantage to collect the distillate in 2-3 fractions. Test the first 5-10 cc. for hydrocyanic acid, chloroform, alcohol, methyl alcohol and possibly for iodoform and nitrobenzene. The other fractions, containing possibly less volatile substances, are used to test for carbolic acid and other phenols volatile with steam, as well as for chloral hydrate, aniline, formaldehyde, phosphorus and carbon disulphide.

Phosphorus.—See page 1. **Phosphorescence in Mitscherlich apparatus** during distillation in a dark room. Distillate, evaporated with strong chlorine or bromine water, or fuming nitric acid, gives phosphoric acid, recognized by **test with ammonium molybdate** or **magnesia mixture**. As an alternative procedure, test original material for phosphorus, as well as phosphorous acid, by **Blondlot-Dusart method**.

Hydrocyanic Acid.—See page 27. **Preliminary test:** blue color on paper strip moistened first with guaiac tincture and then with a little copper sulphate solution. **Prussian blue test.** **Sulphocyanate test.** **Silver nitrate test:** white, flocculent silver cyanide soluble in ammonia.

Carbolic Acid and Cresols.—See pages 43 and 52. **Odor.** Red color with **Millon's reagent** upon warming. Yellowish white, crystalline precipitate of tribromo-phenyl hypobromite with **bromine**

water. Violet color with ferric chloride, passing into yellow upon acidification with mineral acids. **Examine urine**, determining especially sulphate and ethereal sulphuric acid. The latter is greatly increased in phenol poisoning

Thymol.—See page 54 **Odor of thyme.** Distillate frequently contains oil-drops. Extract with ether and evaporate ether. Test only residue for thymol: concentrated sulphuric acid **Piria's**, **Eykman's**, **Liebermann's** and **Lustgarten's** tests. No red color with **Millon's reagent** **Bromine Water** gives only milkiness but no crystalline precipitate (distinction from carbolic acid and other volatile phenols)

Naphthols.—See page 57. Extract distillate with ether and evaporate solvent Test residue for naphthol. **Chlorine water.** **Ferric chloride.** **Piria's test.** **Chloral hydrate test.** **Formaldehyde test.** **Lustgarten's test.** **Millon's test.** **Diazonium test.** Differentiate α - from β -naphthol by **Jorissen's** and **reverse Molisch's test.**

Chloroform.—See page 59 **Odor.** Repulsive odor of **phenyl-isocyanide** upon heating with little aniline and potassium hydroxide solution. Red color upon heating with resorcinol and potassium hydroxide solution. Blue color upon gentle heating with α -naphthol and potassium hydroxide solution Reduction upon warming distillate containing chloroform with ammoniacal silver nitrate solution and **Fehling's solution**

Chloral Hydrate.—See page 64. Gives chloroform reactions. Also aldehyde test with **Nessler's reagent:** Brick-red precipitate, becoming after some time yellow or dirty green. Gives chloroform and magnesium formate when heated with magnesium oxide and water Test for formic acid by warming with silver nitrate or mercuric chloride solution. Occurs in urine, after administration of chloral hydrate, as urochloralic acid.

Butyl Chloral Hydrate.—See page 67. Reacts like chloral hydrate with **Nessler's reagent.** But differs from it in not giving chloroform with caustic alkalies, nor the phenyl-isocyanide test.

Iodoform.—See page 68 **Odor.** Distillate milky, whitish. Ether extract of distillate leaves crystals upon evaporation. Distillate containing iodoform gives chloroform reactions, especially well **Lustgarten's test.**

Aniline.—See page 71. Violet color with calcium hypochlorite solution Repulsive odor of **phenyl-isocyanide**, heated with 1-2

drops of chloroform and potassium hydroxide solution. Flesh-colored precipitate with **bromine water**. Red color on warming with **Millon's reagent** and evolution of nitrogen. **Chromic acid test**.

Nitrobenzene.—See page 69. Oil drops in distillate with yellowish color and characteristic odor. Separated in separating funnel, or better extracted with a little ether. Evaporation of ether leaves nitrobenzene. Reduce by shaking with tin or zinc and little concentrated hydrochloric acid, make alkalkine with potassium hydroxide solution, extract with ether, and test residue from ether for aniline.

Alcohol.—See page 73. Gives **iodoform** upon gentle warming with iodine and sodium hydroxide solution. Odor of **ethyl benzoate** when thoroughly shaken with benzoyl chloride and sodium hydroxide solution. Green color and odor of **acetic aldehyde** when warmed with trace of potassium dichromate and hydrochloric acid. Odor of **ethyl acetate** when warmed with little sodium acetate and concentrated sulphuric acid.

Acetone.—See page 76. Gives **iodoform** even in cold with iodine and sodium hydroxide solution, more slowly with ammonia. **Legal's test**. Dissolves mercuric oxide when shaken with mercuric chloride and excess of alcoholic potassium hydroxide solution. Therefore black zone when clear filtrate is covered with ammonium sulphide. Gives **indigo** with o-nitro-benzaldehyde and sodium hydroxide solution.

Isoamyl Alcohol.—See page 78. Extract distillate with chloroform and evaporate solvent. Oil drops having characteristic odor. Saturated aqueous solution prepared by shaking with little water at 15–16° becomes milky when warmed to about 50°. **Marquardt's test**: odor of isovaleryl aldehyde, and finally of iso-valerianic acid, after standing for some time with little water, dilute sulphuric acid, and potassium permanganate (1:1000). **Uffelmann's test**: violet, or more of reddish blue color, upon addition of freshly prepared solution of methyl violet having green color from hydrochloric acid.

Formaldehyde.—See page 79. Gives **general aldehyde reactions**: precipitation of metallic silver with ammoniacal silver nitrate; separation of red cuprous oxide when heated with Fehling's solution, and gray metallic mercury with Nessler's solution. Red color with alkaline phloroglucinol solution. **Hehner's test**: violet color when warmed with fresh, unboiled milk and hydrochloric acid containing ferric chloride. **Morphine-sulphuric acid**: solution of morphine

hydrochloride in concentrated sulphuric acid; purple-red color by mixing this solution with a mixture of aqueous formaldehyde solution and concentrated sulphuric acid. **Denigès test:** with fuchsine-sulphurous acid in strong sulphuric acid solution; blue to red-violet color, increasing in intensity with time. **Grosse-Bohle-Fincke test:** same color with fuchsine-sulphurous-hydrochloric acid. **Rimini's test:** blue color passing into red when mixed with aqueous solutions of phenyl-hydrazine, sodium nitro-prusside and strong sodium hydroxide solution. **Schryver's test:** fuchsine-like color when mixed with freshly prepared aqueous solutions of phenyl-hydrazine hydrochloride and potassium ferricyanide, and then with concentrated hydrochloric acid. Conversion by ammonia into **hexamethylene tetramine** and detection of latter with (a) mercuric chloride (b) potassium mercuric iodide solution: crystals

Methyl Alcohol.—See page 89. **Oxidation** to formaldehyde by glowing copper spiral, potassium permanganate and dilute sulphuric acid, potassium dichromate and sulphuric acid, sodium persulphate and dilute sulphuric acid, or platinum black, and detection of formaldehyde by color reaction. **Autenrieth's test:** shake aqueous solution warmed to about 40° with 10 per cent. sodium hydroxide solution and p-bromo-benzoyl chloride; formation of methyl ester of p-bromo-benzoic acid, appearing in fine crystals from alcohol, methyl alcohol, or acetone, having anise-like odor, and melting at 78°; percentage of bromine may also be determined.

Carbon Disulphide.—See page 100. Black color, or black precipitate (PbS), when heated with little lead acetate and potassium hydroxide solution. Formation of ammonium sulphocyanate by evaporation with concentrated ammonia, detected with little ferric chloride after acidification with dilute hydrochloric acid. Formation of potassium xanthogenate, when shaken with alcoholic potassium hydroxide solution, and detection with copper sulphate solution.

SYNOPSIS OF GROUP II

Examination for Organic Poisons Non-volatile with Steam from Acid Solution but Extracted from Material by Alcohol Containing Tartaric Acid. Stas-Otto Method

Material as finely comminuted as possible acidified with tartaric acid, extracted warm under reflux for 20–30 minutes with twice to three times quantity of pure alcohol with occasional shaking, and

filtered. Filtrate evaporated upon water-bath to thin syrup. Latter stirred with water (100-200 cc.), filtrate again evaporated upon water-bath, and residue, while well-stirred, mixed with quite a large quantity of absolute alcohol, to precipitate proteins and other substances as completely as possible. Filtered alcoholic liquid gives upon evaporation residue that dissolves in 50-60 cc of water usually giving clear or only slightly milky solution that has yellow to brownish color and acid reaction. Acid solution thoroughly and repeatedly extracted direct with ether (A), then after it has first been rendered alkaline with sodium hydroxide solution (B) then also with ammonia (C), and latter alkaline liquid finally with warm chloroform, or with chloroform containing 10 per cent. alcohol (D). Residues left by distilling or evaporating solvents examined according to directions given from A to D. See pages 108 to 225.

A Residue from Ether Extract of Aqueous Tartaric Acid Solution May Contain :

Picrotoxin.—See page 111. **Very bitter.** Reduces Fehling's solution when warmed. **Melzer's test:** red streaks radiating from picrotoxin covered with alcoholic benzaldehyde solution and concentrated sulphuric acid. Concentrated sulphuric acid gives yellow or orange-red color, drop of potassium dichromate solution has brown margin. Mixture of picrotoxin and about three times the quantity of potassium nitrate moistened with trace of concentrated sulphuric acid acquires red color with excess of saturated sodium hydroxide solution.

Colchicin.¹—See page 115. **Very bitter,** yellowish, amorphous. Few drops of mineral acid render aqueous colchicin solutions **intensely yellow.** Dissolves in concentrated nitric acid with dirty violet color, changing at once to brown-red and finally to yellow. Addition of excess of potassium hydroxide solution imparts orange-yellow or orange-red color to mixture. **Zeisel's test:** Heat to boiling yellow solution of colchicin in concentrated hydrochloric acid for 2-3 minutes in test-tube with 2 drops of ferric chloride solution. Green or more of an olive-green color appears upon cooling, especially after addition of about same volume of water. **Fühner's test.**

Picric Acid.—See page 119. **Very bitter,** yellow, usually amorphous from ether solution. In presence of picric acid, material and

¹ Because of its slight solubility in ether, colchicin appears in ether extract of the tartaric acid solution only in small quantity. Most of it is found in the chloroform extract of the solution rendered alkaline with ammonia (see D).

various extracts have more or less intensely **yellow color**. **Isopurpuric acid test**: Aqueous picric acid solution gently warmed with few drops of saturated potassium cyanide solution becomes red. Similarly aqueous picric acid solution becomes red when warmed with little ammonium sulphide. Aqueous picric acid solution dyes wool and silk yellow, but not cotton.

Acetanilide.—See page 122. Faint, burning taste. **Indophenol test**: Acetanilide, boiled down to about 20 drops with few cc of concentrated hydrochloric acid and treated when cold with aqueous phenol solution and calcium hypochlorite solution added drop by drop, gives when shaken dirty red to violet color, becoming deep blue when covered with ammonia. Acetanilide heated to boiling first with alcoholic potassium hydroxide solution and again after addition of little chloroform gives repulsive odor of **phenyl-iso-cyanide**.

Phenacetine.—See page 125. Tasteless. Gives **indophenol** but not **phenyl-isocyanide test**. Concentrated nitric acid colors phenacetine yellow and dissolves it with yellow to orange-red color. Heated with dilute nitric acid, gives yellow or orange-yellow solutions. If these solutions are saturated, nitro-phenacetine appears as yellow crystals as solutions cool.

Salicylic Acid.—See page 131. Usually needle-like crystals, having sweetish, acidulous, rather harsh taste. Drop of **ferric chloride** solution colors aqueous solution blue-violet, in greater dilution red-violet. Red color when warmed with **Millon's reagent**. **Bromine water** in excess produces a yellowish white, crystalline precipitate.

Veronal.—See page 137. Bitter, crystallizes and sublimes easily. If solution of ether extract in as little sodium hydroxide solution as possible, sodium carbonate solution, or aqueous ammonia is filtered and filtrate acidified with dilute hydrochloric acid, veronal will crystallize. **Melting-point** ($187-188^{\circ}$) of dry crystals, as well as that of a mixture of these with absolutely pure veronal, may be determined. It should be the same in both cases. With **Millon's reagent** saturated, aqueous veronal solution gives white, gelatinous precipitate soluble in large quantity of reagent. When shaken this solution foams like soap solution.

Antipyrine.¹—See page 141.—Mildly bitter taste. Test aqueous solution of residue for antipyrine: Red color with drop of **ferric**

¹ Most of the antipyrine when present is found in ether extract B of the aqueous alkaline liquid as well as in chloroform extract D

chloride solution. Green color with 1-2 drops of fuming nitric acid. If solution is boiled and another drop of fuming nitric acid added, green color changes to red.

Caffeine ¹—See page 145. Faintly bitter. Evaporated upon the water-bath with saturated chlorine water, caffeine gives red-brown residue. Moistened with very little ammonia, becomes purple-violet. Good procedure (E. Fischer) to boil substance in test-tube with strong chlorine water, or with hydrochloric acid and trace of potassium chlorate, evaporate to dryness in dish upon water-bath, and moisten residue with ammonia.

B. Residue from Ether Extract of Aqueous Solution Rendered Alkaline with Sodium Hydroxide Solution May Contain:

Coniine.—See page 153. Yellow oil drops with penetrating odor. Cold, saturated aqueous solution milky when warmed. Spontaneous evaporation of trace of coniine with drop of hydrochloric acid leaves coniine hydrochloride as doubly refractive crystals that are needle or prism-shaped and sometimes in star-like clusters. **Physiological test:** Paralysis of peripheral nerves; action similar to that of curare.

Nicotine.—See page 156. Liquid. Remains dissolved in residual water upon evaporation of ether and has faint tobacco odor. **Melzer's test:** Red color when heated with 2-3 cc of epichlorohydrine. **Schindelmeiser's test:** Intense red color after standing several hours with drop of formaldehyde solution upon addition of drop of nitric acid. **Roussin's test:** Ether solution of iodine after long standing with nicotine in closed test-tube gives ruby-red, crystalline needles.

Aniline.—See page 71. Reddish or brownish oil drops upon evaporation of ether extract. Test aqueous solution of these as directed above for aniline (see page 160).

Veratrine.—See page 160. Soluble in concentrated sulphuric acid² with yellow color gradually passing into orange, red and finally cherry-red. Gentle heat favors this color change. Solution of vera-

¹ Caffeine behaves like antipyrine. Ether removes only a small part from the tartaric acid solution. The greater part of antipyrine when present will be found in the ether extract B and mainly in the chloroform extract D.

² Before a definite conclusion is drawn from the result and course of a reaction with concentrated sulphuric acid, concentrated nitric acid, and the reagents of Erdmann, Froehde, Marquis and Mecke, a careful, comparative study of the behavior of these reagents with the various alkaloids should first be made (Pages 237 to 238.)

trine in concentrated sulphuric acid shows at first pronounced green-yellow fluorescence. Froehde's reagent produces same color changes. Stable red color when warmed in water-bath with concentrated hydrochloric acid. **Weppen's test:** Mixture of veratrine with about 6 times quantity of cane-sugar gradually colored green and finally blue with few drops of concentrated sulphuric acid. Concentrated sulphuric acid containing furfural may be substituted for cane-sugar.

Strychnine.—See page 164. Frequently very fine, intensely bitter, crystalline needles upon evaporation of ether solution. Small particle of potassium dichromate colors sulphuric acid solution of strychnine fugitive blue or blue-violet. Mandelin's reagent produces same color but more stable than that given by potassium dichromate.

Brucine.—See page 171. Concentrated nitric acid dissolves brucine with blood-red color changing immediately to red-yellow and yellow. Stannous chloride solution added drop by drop to yellow solution in test-tube converts yellow to violet. Or solution of brucine in very dilute nitric acid may be carefully added as upper layer to concentrated sulphuric acid. Result is red or red-yellow zone.

Atropine.—See page 173. Evaporated in porcelain dish upon water-bath with few drops of fuming nitric acid, atropine remains as yellowish residue that becomes violet when moistened with alcoholic potassium hydroxide solution. Hyoscyamine and scopolamine also give this test. Behavior of strychnine and veratrine similar to that of atropine. **Physiological test with cat's eye:** Even drop of atropine solution diluted 1:130,000 produces dilatation of pupil. **Gulielmo's odor test.** **Micro-reactions of Eder.**

Cocaine.—See page 185. Precipitated from solutions of salts by potassium hydroxide solution as oil drops that soon solidify and become crystalline. **Permanganate test.** **Iodic acid test.** **Detection of benzoyl-group:** If cocaine is warmed in test-tube with 1 cc. of concentrated sulphuric acid for 5 minutes in boiling water-bath and 2 cc. of water are then carefully added, odor of methyl benzoate will be recognized and as solution cools crystals of benzoic acid will appear. Latter may be identified by melting-point (120°) and tendency to sublime. **Physiological test:** Anaesthesia of the tongue.

Codeine.—See page 197. Concentrated sulphuric acid dissolves codeine in cold without color; after standing for some time or at once

when gently warmed, solution takes on reddish or more of bluish color. Solution of codeine in concentrated sulphuric acid gently warmed with potassium arsenate, or instead of the latter with trace of ferric chloride solution, becomes pure blue or blue-violet. Soluble in Froehde's reagent with yellowish color, changing at once to green and upon gentle warming to blue. Codeine dissolves in formalin-sulphuric acid with reddish violet color, immediately changing to stable blue-violet. Codeine gives Pellagri's test (see Morphine).

Narcotine.—See page 201. Reaction not alkaline (distinction from other alkaloids) and taste not bitter. Soluble in Froehde's reagent with green color. If concentrated Froehde's reagent is used (0.05 gram of ammonium molybdate + 1 cc. of concentrated sulphuric acid), color greenish at first gradually changes to fine cherry-red and from the margin to blue. Soluble in Erdmann's reagent with fine red color.

Hydrastine.—See page 205. Soluble in Froehde's reagent with quite stable green color that changes later to brown. Dissolves in Mandelin's reagent with red color, gradually changing to orange-red. Solution of hydrastine in dilute sulphuric acid, shaken with very dilute potassium permanganate solution, exhibits intense blue fluorescence. Permanganate solution should be added drop by drop.

Quinine.—See page 209. Residue from ether solution usually amorphous varnish having very bitter taste. Solution of quinine in dilute sulphuric acid exhibits blue fluorescence. **Thalleoquin test:** About 1 cc. of strong chlorine water first added to solution of residue in dilute acetic acid and then at once drop by drop ammonia in excess, emerald-green color appears, if quinine present. **Hera-pathite test:** Solution of residue from ether in about 10 drops of mixture of 30 drops of acetic acid, 20 drops of absolute alcohol, and 1 drop of dilute sulphuric acid, heated to boiling and treated with 1 drop of alcoholic iodine solution (1:10). If quinine present, lustrous olive-green leaflets, having cantharides-green appearance in reflected light, separate. **Erythro-quinine test:** One drop each of half-saturated bromine water, potassium ferrocyanide solution (1:10) and ammonia added to 10 cc. of faintly acid, preferably acetic, solution of residue produce with agitation gradually red color. If this mixture is extracted at once with chloroform, this solvent will take on fine violet-red color if quinine present.

Pyramidone.—See page 216. Frequently obtained as fine needles easily soluble in water by evaporating ether solution. Acidified

aqueous solution gives fugitive blue-violet color with little potassium nitrite, this color quickly disappears upon further addition of nitrite. Blue-violet color with ferric chloride solution, or very dilute iodine solution, soon becoming red-violet and then fading. Blue color with silver nitrate solution, later darkening with separation of metallic silver

Antipyrine and Caffeine.—Detected by means of tests given under A

Physostigmine.—See page 191 Evaporated with ammonia gives blue residue soluble in alcohol with blue color **Physiological test:** Contraction of the pupil.

C¹ Residue from Ether Extract of Aqueous Solution² Rendered Alkaline with Ammonia May Contain:

Apomorphine.—See page 219 Residue amorphous and usually greenish Drop of concentrated nitric acid colors solution in little concentrated sulphuric acid fugitive violet, then red-yellow or orange. Soluble in Froehde's reagent with green or violet color. If solution of apomorphine in dilute hydrochloric acid first treated with excess of sodium bicarbonate and then well-shaken with 1-2 drops of alcoholic iodine solution, it acquires blue-green or emerald-green color, and ether shaken with it takes on violet color **Wangerin's test:** Solution of apomorphine hydrochloride shaken with 1-2 drops of potassium dichromate solution (0.3 per cent $K_2Cr_2O_7$) gradually turns dark green, and if chloroform added it becomes violet. Upon careful addition of dilute stannous chloride solution, chloroform takes on pure indigo-blue color. **Feinberg's test.**

D Residue from Chloroform Extract of Aqueous Solution Rendered Alkaline with Ammonia, or Sodium Bicarbonate, May Contain:

Morphine.—See page 225. Very bitter, usually amorphous, rarely crystalline, from chloroform solution. **Preliminary test:** Test-portion of residue dissolved in little dilute sulphuric acid, treated with few drops of iodic acid solution, and shaken with chloroform, latter will have violet color. Soluble in Froehde's reagent with

¹ Extraction with ether according to C is necessary only when aqueous solution rendered alkaline with sodium hydroxide solution is deep green and supernatant ether solution is rose or red-violet, that is, when apomorphine is present

² Aqueous solution from B alkaline from sodium hydroxide, after separation of ether extract, is first acidified with dilute hydrochloric acid (test with litmus paper), then rendered alkaline with ammonia, or sodium bicarbonate, and at once thoroughly extracted repeatedly with ether, if necessary, or direct with hot chloroform, or with a hot alcohol-chloroform mixture (1 + 9).

violet color, gradually changing to dirty green and finally to faint red. Soluble in Marquis' reagent with purple-red color, later becoming blue-violet and almost pure blue. **Husemann's test:** Solution of morphine in concentrated sulphuric acid, heated over very small flame until copious fumes of sulphuric acid appear, gives when cold with drop of concentrated nitric acid fugitive red-violet color changing at once to blood-red or red-yellow. **Pellagri's test.** Ferric chloride test: Not too little of the residue from chloroform dissolved in few drops of very dilute hydrochloric acid, solution evaporated upon water-bath to dryness, residue dissolved in little water, and drop of ferric chloride solution added. Appearance of blue color indicates presence of morphine. **Biological test.**

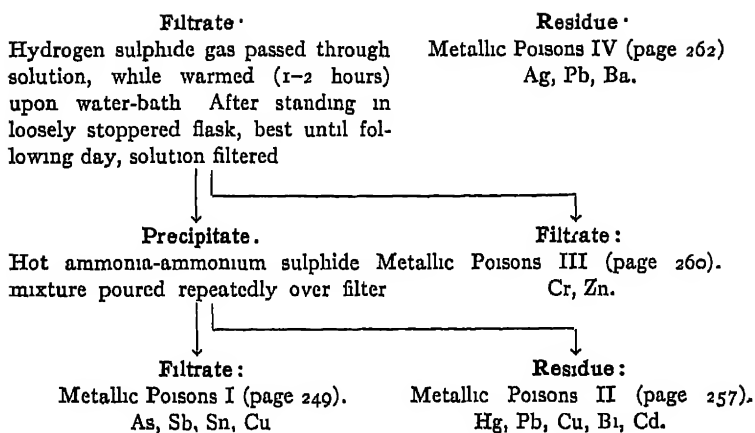
Narceine.—See page 234. Blue color with iodine water. Intensely yellow solution in resorcinol-sulphuric acid exhibits carmine-red, often more of cherry-red, color when warmed upon water-bath and stirred. Yellow-brown solution of narceine in tannin-sulphuric acid becomes pure green when warmed upon water-bath.

Colchicin.—Yellow or more of brownish yellow varnish giving very well tests described for colchicin (see A).

Antipyrine and Caffeine.—These two substances relatively difficultly soluble in ether, but easily soluble in chloroform, also frequently occur, if present, in residue from chloroform extract D and may be recognized by tests described under A.

SYNOPSIS OF GROUP III

Residue left after volatile poisons (Group I) have been distilled off, or portion of original material, is stirred in a glass flask or porcelain dish with dilute hydrochloric acid (10 per cent. HCl). Solid potassium chlorate, or its concentrated aqueous solution, is added and mixture is heated upon boiling water-bath and frequently stirred until most of material has dissolved and liquid has yellow color. Few drops of sulphuric acid are added to solution after dilution with water and it is filtered when cold. In case filtrate contains much free acid, most of excess is removed by evaporation.



CHAPTER IV

POISONS NOT IN THE THREE MAIN GROUPS

A. INORGANIC SUBSTANCES

Mineral Acids

As a rule, examination of cadaveric material for mineral acids is necessary only when the autopsy points conclusively to such poisoning. That is, when characteristic corrosions and discolorations about the face, mouth, oesophagus and stomach are visible. Since chlorides, nitrates and sulphates are normal constituents of nearly all animal organs and fluids, proof must be given that free mineral acid is present

To detect free mineral acid, extract a portion of material with cold water, filter and test as follows, if the solution is strongly acid:

1. **Methyl Violet Test.**—Add a few drops of an aqueous (0.1:1000), or alcoholic (1:100), solution of methyl violet to a portion of the filtrate. Free mineral acid produces a blue or green color. Organic acids are without action

2. **Methyl Orange Test.**—Add a few drops of dilute aqueous solution of methyl orange to a portion of the filtrate. A red color indicates free mineral acid.

3. **Congo Paper Test.**—Even very dilute solutions of free mineral acids turn "Congo paper" blue. Organic acids are without action.

4. **Günzburg's Test.**—Mix a few drops of the filtrate with 3-4 drops of Günzburg's reagent¹ and evaporate to complete dryness upon the water-bath, or over a small flame. Free hydrochloric or sulphuric acid gives a fine red or reddish yellow residue. Nitric acid gives more of a yellowish red residue.

If general tests show presence of free mineral acid, make special tests for the particular acid

Hydrochloric Acid

1. **Chlorine Test.**—Warm a little of the aqueous extract, not too dilute, with finely powdered manganese dioxide. Free hydrochloric

¹See "Preparation of Reagents," page 642

acid yields chlorine, recognized by color and odor, or by passing the gas into potassium iodide solution and liberating iodine. Hydrochloric acid exclusively does not give this test. A chloride and free sulphuric acid give chlorine under the same conditions. Free bromine will also liberate iodine from potassium iodide.

2. **Distillation.**—If possible, separate hydrochloric acid from other substances by distillation and test the distillate. Concentration of the acid is especially important, since dilute hydrochloric acid upon distillation at first yields only water. Hydrochloric acid¹ itself does not begin to distil until concentration is about 10 per cent. Since dilute hydrochloric acid is usually examined, distil the material mixed with water, or preferably a filtered aqueous extract, nearly to dryness. In such distillation apply heat by means of an oil-bath. To detect hydrochloric acid in the distillate, acidify with dilute nitric acid and add silver nitrate solution. Frequently a quantitative estimation of hydrochloric acid is required. In absence of other acids, titrate the distillate with 0.1 N-sodium hydroxide solution, using phenolphthalein as indicator. Otherwise, estimate the acid gravimetrically, precipitating with silver nitrate and weighing silver chloride, or volumetrically by Volhard's method. Since the human stomach normally contains 0.1-0.6 per cent of free hydrochloric acid, examination of stomach-contents for this acid must always include a quantitative estimation. A conclusion that poisoning by hydrochloric acid has taken place is justifiable only when a large quantity of acid is found, provided also that the autopsy unmistakably indicates its presence.

Nitric Acid

The human body normally contains only a very small amount of nitrates. When present, they owe their origin usually to drinking water and vegetable foods. Human urine almost always shows traces of salts of nitric and nitrous acids. Chemical examination of cadaveric material need not include tests for nitric acid, unless the autopsy affords evidence of poisoning by this acid, such as distinct signs of corrosion about lips, mouth, oesophagus and stomach and sometimes perforation. These parts are more or less yellow or yellowish brown. Yellow froth is said to exude from the mouth

¹ If, for example, 100 cc. of 1 per cent. hydrochloric acid are distilled, the first 90 cc of distillate will contain only traces of acid. Almost all acid will be found in the last portion of distillate.

and nose of the cadaver. Also stomach-contents are yellow in concentrated nitric acid poisoning. If concentration of acid is less than 20 per cent, these specific changes may not appear in the gastrointestinal tract. Nitric acid taken internally, dilute or concentrated, appears at once in the urine.

Detection of Nitric Acid

1. Distillation.—If possible, extract material direct with water, filter and test the filtrate for nitric acid in the usual way. When the quantity of nitric acid is large, separate it from other substances by distilling the filtered aqueous extract. Apply heat by means of an oil-bath. Like hydrochloric acid, nitric acid¹ does not distil until a definite concentration is reached. The filtered extract should be distilled nearly to dryness. Considerable nitric acid is lost, for it combines with organic substances, particularly proteins, forming nitroderivatives, xanthoproteic acid, etc. Nitric acid may also cause oxidation. So the distillate will not contain all the acid originally present. Toward the end of distillation brown vapors of nitrogen peroxide often appear. Such a distillate, added to starch paste and potassium iodide, produces an immediate blue color in presence of dilute sulphuric acid. The residue from distillation is usually distinctly yellow.

The following tests serve to detect nitric acid in the distillate:

2. Fleury's Procedure.²—Nitric acid, according to Fleury, varies in effect upon the human body. Sometimes it stains epithelial tissue yellow, having merely a superficial action; again in contact with muscle tissue it forms an eschar, or converts it into a cheesy mass through coagulation of albumin.

Positive detection of nitric acid poisoning is more difficult than generally supposed. To detect free nitric acid still present unchanged, extract the finely divided material with three times the quantity of alcohol, filter and add slaked lime in excess to the filtrate. To decompose any nitric acid ester present, allow the mixture to stand for 12 hours, filter and evaporate the filtrate to dryness. Dissolve the residue in 95 per cent. alcohol, expel alcohol from the filtered

¹ If a thin magma of crumbled dog-biscuit and 100 cc. of 1 per cent. nitric acid are distilled, by far the greater part of acid will be found in the last 10 cc. of distillate.

² G. Fleury. *Toxicological Detection of Nitric Acid*. *Ann. Chim. analyt. appl.* 6 (1901), 12; *Rep. de Pharm.* 56 (1907), 385.

solution, and finally test an aqueous solution of the residue for nitric acid. By this method Fleury obtained about 20 per cent. of the nitric acid from animal material. This procedure converts the acid into the calcium salt which is soluble in 95 per cent alcohol. But sodium nitrate is also quite soluble (1.50) in alcohol of the same strength. So if the final residue gives a faint test for nitric acid, proof of free acid in the original material is not conclusive. The method of Baumert obviates this difficulty.

3. Baumert's Procedure.¹—Neutralize the material itself, or its aqueous extract, with milk of lime, dry and extract with alcohol; or, after neutralization with milk of lime or calcium carbonate, evaporate to a syrup, stir and mix with alcohol. Distil the filtered alcoholic extract obtained in either way, dissolve the residue in water, filter and evaporate the solution. Dissolve the residue again in alcohol and allow this solution to stand for several hours in a closed flask with about the same volume of ether. Filter this alcohol-ether solution, evaporate the solvent, and dissolve the residue in a little water. Apply the following nitric acid tests to this solution.

(a) **Diphenylamine-Sulphuric Acid Test.**—Add a few drops of diphenylamine sulphate solution² to the aqueous extract, or distillate, and carefully pour this mixture upon pure concentrated sulphuric acid free from nitric acid. If nitric acid is present, a blue zone appears where the two liquids meet.

(b) **Brucine-Sulphuric Acid Test.**—Mix the same volume of brucine sulphate solution³ with the liquid to be tested and carefully pour this mixture upon pure concentrated sulphuric acid. If nitric acid is present, a red zone appears where the two liquids meet.

(c) **Ferrous Sulphate-Sulphuric Acid Test.**—Saturate the liquid to be tested with pure ferrous sulphate and carefully pour this solution upon concentrated sulphuric acid. If nitric acid is present, a black zone appears where the two liquids meet.

¹ G. Baumert *Lehrbuch der gerichtlichen Chemie* Second edition, 1907.

² Solution prepared from 1 gram of diphenylamine, 5 grams of dilute sulphuric acid, and 100 cc. of water.

³ Solution prepared from 1 gram of brucine, 5 grams of dilute sulphuric acid, and 100 grams of water. The sulphuric acid used in preparing both these reagents should not itself give the test. Should it do so, the sulphuric acid must be heated in a platinum dish until nitrous acid is completely expelled, or the acid may be distilled from a small retort, when nitric and nitrous acid will be found in the first part of the distillate which should be rejected.

(d) **Copper Test.**—Place a small piece of clean copper (wire or sheet) in nitric acid and heat. Red-brown vapors of nitrogen peroxide (NO_2) appear.

Sulphuric Acid

Nearly all animal and vegetable substances normally contain sulphates. So an examination for free sulphuric acid must exclude its salts. It is useless to examine cadaveric material for free acid, unless marked corrosion and discoloration of lips, mouth, oesophagus, and stomach indicate its presence. There are eschars upon the lips and the mucous lining of the mouth is grayish white. The white coating on the back of the tongue may have been dissolved, exposing the firm, brownish muscular tissue beneath. The tongue often looks as if it had been boiled. The mucous lining of the oesophagus is much wrinkled and coated gray. Externally the stomach is usually brown, or slate-gray, and its contents black. Frequently in sulphuric acid poisoning there is perforation of the stomach wall and brownish black masses find their way into the abdominal cavity. There may be black spots in the stomach, due according to R. Kobert (Intoxikationen) not to charring, as previously supposed, but to brown-black haematin. Acids decompose the blood-pigment oxyhaemoglobin mainly into haematin and protein (globulin). Methaemoglobin and haematoporphyrin may also be formed. Acids produce the latter from haematin and in the change there is loss of iron. All three of these decomposition products of the red blood-pigment, namely, methaemoglobin, haematin and haematoporphyrin may be formed successively and then appear in the urine. The blood in the stomach walls is often acid and then contains chiefly methaemoglobin and haematin. The mucosa of the intestines even far down may be grayish white and strongly acid.

Detection of Sulphuric Acid

1 Extract the finely divided material, if strongly acid, with cold absolute alcohol and after some time filter. The solution contains sulphuric acid but not sulphates. Evaporate the alcoholic filtrate upon the water-bath, or, if the volume is large, distil the alcohol. Dissolve the residue in 10 cc. of water and heat the solution to boiling to saponify ethyl sulphuric acid. Filter and test the filtrate with barium chloride or lead acetate solution. To prove that the precipitate is a sulphate, mix with sodium carbonate and fuse upon charcoal. Sodium sulphide formed blackens metallic silver in presence of water, or gives hydrogen sulphide with acids.

2 Extract the finely divided material with water and apply the following tests to the filtrate.

(a) **Sugar Test.**—Evaporate some of the filtered extract in a porcelain dish with a small particle of sugar. Free sulphuric acid produces a black, carbonaceous residue.

(b) **Sulphur Dioxide Test.**—Concentrate the filtered extract upon the water-bath and heat in a test-tube with a few pieces of copper. Free sulphuric acid generates sulphur dioxide, recognized by its stifling odor. Distil sulphur dioxide (preferably in an atmosphere of carbon dioxide) into a little water and test the distillate as follows:

α Warm a portion of liquid with a little stannous chloride solution. A yellow precipitate of stannic sulphide¹ appears.

β Add iodo-potassium iodide solution drop by drop. The color of iodine disappears and at the same time sulphuric acid is formed. Barium chloride then precipitates barium sulphate insoluble in dilute hydrochloric acid.

To estimate sulphuric acid quantitatively, either precipitate and weigh barium sulphate in the usual way, or titrate with 0.1 N-sodium hydroxide solution, using phenolphthalein as indicator.

1000 cc. of 0.1 N-sodium hydroxide solution = 0.1 gram-equivalent of sulphuric acid = 4.9 grams of H_2SO_4 . In the latter method no other acid except sulphuric should be present.

Hydrofluoric Acid and Soluble Fluorides

Hydrofluoric acid is a powerful, corrosive poison. In producing toxic phenomena due to corrosion and acidification, its vapor is quite as effective as that of hydrochloric acid. Of water soluble, metallic fluorides, sodium fluoride in particular has been tested thoroughly as to toxic action. This salt finds use in medicine. Tappeiner has found sodium fluoride a protoplasm poison. Very likely it acts by withdrawing calcium. Applied to the cornea, it produces ulcers, subcutaneous necrosis and suppuration. Injected into the blood, it paralyzes the central nervous system, causing at the same time muscular twitching and convulsions. Coagulation of blood is also prevented, for calcium needed in this reaction is precipitated through combination with fluorine. Taken by the mouth, sodium fluoride may cause inflammation of the mucous lining of the stomach. Action of the heart is weakened and blood-pressure falls. Death finally takes place from paralysis of the respiratory centre. Sodium fluoride exerts a strong antiseptic action upon bacteria. Baldwin² cites the case of several persons made very ill from eating cake in the preparation of which sodium fluoride was used by mistake for baking powder. The person who had eaten most of the cake died on the same day.

To detect water-soluble fluorides in such material as gastric or intestinal contents, extract with water, filter and precipitate with calcium chloride. Collect the precipitate upon a filter, dry (best by gentle ignition in a crucible), and make the following tests for fluorine.

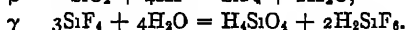
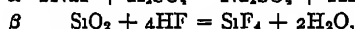
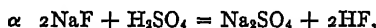
(a) Etching Test.—Pour a little concentrated sulphuric acid upon some of the dried precipitate in a platinum crucible. Cover with a watch-glass coated upon one side with paraffine and then written upon. Allow the watch-glass to remain over the crucible for about one hour. Then remove the paraffine coating. Etching of glass exposed by the writing shows presence of fluorides.

¹ Sulphurous acid and sodium sulphite, added to stannous chloride solution not too strongly acid, precipitate stannous sulphite, $SnSO_3$, white and readily soluble in hydrochloric acid. Warmed in presence of hydrochloric acid, sulphur dioxide acts upon a stannous salt as an oxidizing agent. A precipitate of $Sn_2O_3 \cdot S_2$ is formed, or H_2S is evolved and $SnCl_4$ formed, depending upon the amount of hydrochloric acid present. (Prescott and Johnson, Qualitative Chemical Analysis. Fifth edition, page 86.)

² H. B. Baldwin. Journ. of the Amer. Chem. Soc. 21, 517.

(b) **Silicic Acid Test.**—Mix the well-dried precipitate with a little sand and heat with concentrated sulphuric acid in a dry test-tube. Bring a glass rod having on its end a drop of water in contact with the vapor given off, or pass the vapor into a larger moist test-tube. In presence of hydrofluoric acid the drop of water solidifies because of separation of silicic acid more or less gelatinous, and a white efflorescence also consisting of silicic acid forms upon the moist part of the test-tube.

Explanation of Reaction.—Hydrofluoric acid set free from the fluoride by sulphuric acid dissolves the sand forming gaseous silicon tetrafluoride. Water decomposes the latter into silicic acid, which separates gelatinous, and soluble fluosilicic acid,



Gelatinous In solution
[precipitate

Official Directions (German) for Detection of Hydrofluoric Acid and Salts in Meat

Thoroughly mix 25 grams of finely divided meat in a platinum dish with sufficient milk of lime. Then dry, ash and transfer the powdered residue to a platinum crucible. Moisten the powder with 3 drops of water and add 1 cc. of concentrated sulphuric acid. After adding the sulphuric acid, at once cover the crucible with a large watch-glass and heat upon an asbestos plate. The convex side of the watch-glass should be covered with paraffine and the glass exposed by writing. Put a piece of ice in the watch-glass to keep the paraffine from melting. If the glass exposed by the writing is etched, presence of hydrofluoric acid is proved and the meat is regarded as having been treated with hydrofluoric acid or fluorides.

Bromine and Alkali Bromides

Free bromine or its vapor colors the skin brown and may cause blisters and necrosis. It corrodes mucous membranes causing intense burning pain. No matter where applied, bromine after absorption gives rise to nervous depression, stupor and narcosis. Bromine vapor inhaled causes inflammation of the conjunctiva, lachrymation, coryza, salivation, distressing attacks of coughing, pseudo-asthma and bronchitis. At the same time there may be abdominal pains, diarrhoea and vomiting. The most frequent manifestations are giddiness, headache and stupor (R. Kobert). Severe disturbances of a general character may result from long-continued use of large quantities of alkali bromides (sodium, potassium or ammonium bromide). Chronic bromism manifests itself in coryza, coughing, heaviness in the head, weakening of the mental faculties, disturbance of speech, sexual impairment, but particularly in skin eruptions (bromine acne) which may become malignant. The secondary effects due to alkali bromides resemble in a way those caused by potassium iodide but are ordinarily more persistent than the latter. Bromides attack mucous membranes more easily than iodides do. Moreover the intensity and duration of the effect of bromides upon the skin, and in still higher degree upon the central nervous system, are greater than in the case of iodides. As a rule, the range of tolerance of alkali bromides is not very wide.

Resistance of children 8-14 years of age, as a rule, is said to be higher than that of adults. Individuals have used alkali bromides for years without appearance of secondary effects. But in other cases these have appeared after very small quantities have been taken. Lewin¹ cites the case of a man who took 2 grams of potassium bromide and lapsed into a state in which he believed he dreamed everything he saw, heard and felt. The way in which absorption takes place is immaterial. Whether introduced by way of the stomach, by inhalation, or by the rectum bromides may give rise to secondary effects.

Alkali Bromides in the Human Body

Absorption of alkali bromides from the mucous membranes is rapid. But elimination is much slower. The human body stubbornly retains sodium bromide taken by the mouth and eliminates it through the kidneys very slowly.

In an experiment by Autenrieth,² an adult with healthy kidneys, non-epileptic, on a mixed diet, and with continuously good diuresis, took two doses of 4 grams each of sodium bromide and eliminated in the course of 35 days 5.68 grams in the urine, or 71.8 per cent. On the 33d day after the last dose 10 mg. of sodium bromide were found in the 24 hour urine. In this as well as in other experiments it was found that very little sodium bromide, only 3.2 per cent. of that taken, was eliminated on the first two days after medication. Elimination attained its maximum on the third day. The ability of two persons with diseased kidneys to eliminate sodium bromide, as compared with the person having healthy kidneys, was far less. The fact, however, must be taken into consideration that the two persons with diseased kidneys were on a diet low in salt. An instance given by Autenrieth³ is in full agreement with these experimental results. Parts of the cadaver of a man 37 years old, ill from chronic nephritis, were examined. He had been on a diet low in salt and had taken daily for 10 days at least 15 grams of sodium bromide. Then during 28 days prior to death he had taken no bromide. After this long period without bromide the blood and different organs of this man had retained a considerable quantity, that is, liver 0.037, brain 0.019, kidneys 0.037, and blood 0.045 per cent. of bromine calculated as sodium bromide, and for the organ containing all its blood. Even after large quantities of sodium bromide have been taken the brain does not show an increase in bromine.

Detection and Estimation of Bromine

To detect free bromine, for example, in stomach-contents, or in organs of the body, the material should be first distilled with water. A case, however, where this is necessary would rarely occur, for free bromine would at once combine chemically with the organic substances forming bromine substitution products and hydrobromic acid. Should simple distillation fail to show bromine, add

¹ L. Lewin. "Secondary Effects of Drugs." Third edition, 1899, page 157.

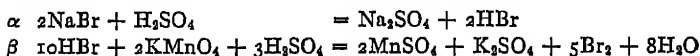
² W. Autenrieth. Elimination of Bromine from Man following Administration of Sodium Bromide. Münch. med. Wochenschr. 1918, page 749.

³ W. Autenrieth. Estimation and Distribution of Bromine in Organs and Blood Following Administration of Sodium Bromide. Münch. med. Wochenschr. 1918, page 33.

excess of potassium bichromate to the material, render strongly acid with dilute sulphuric acid, and distil again. But even this procedure is not always successful. In the experiments mentioned above, Autenrieth found a fusion method advantageous. He tested for bromine qualitatively and at the same time determined it quantitatively by the colorimetric method.

Ashing of Urine and Colorimetric Estimation of Bromine in Ash

Estimation of bromine colorimetrically in alkali and other bromides depends upon the reaction taking place in presence of potassium permanganate and dilute sulphuric acid, or better acid potassium sulphate solution. Bromine is completely set free and taken up without loss in a measured volume of chloroform. By means of a calibrated comparison-wedge the tinctorial strength of this solution is determined in the Autenrieth-Königsberger colorimeter.¹



Procedure.—According to the probable quantity of bromine present, take 20, 50, 100 or more cc of urine. Add 1 gram of sodium carbonate and 0.5-1 gram of potassium nitrate and evaporate the measured volume to dryness in a rather deep nickel crucible of sufficient capacity to be only half-full. Near the end of evaporation heat the crucible cautiously with a smaller flame, to avoid loss from spitting due to too vigorous deflagration. Application of heat on the side rather than bottom of the crucible is advisable. In this manner urine may be quickly evaporated without frothing over. Further heating after sprinkling a little finely powdered potassium nitrate over the dry residue, best while rotating the crucible, will remove any particles of carbon and bring about complete fusion of the mass. Of course the melt should not be heated too intensely nor needlessly long. But even then there is hardly any loss of sodium bromide, for its vapor tension is low and any bromide in the vapor phase is at once precipitated on the cooler parts of the deep covered crucible. Usually 2-3 grams of potassium nitrate are enough for complete combustion of carbon particles in the ash from 100 cc of urine. Soften the cold melt with 10-15 cc of water (sodium bromide is so soluble that more water is not needed) and neutralize the solution in the crucible with dilute sulphuric acid so that red litmus paper is turned distinctly blue. Filtration of the usually turbid solution is not necessary. Transfer to a separating funnel and without loss rinse the crucible with 10-20 cc of 10 per cent. acid potassium sulphate solution. According to the probable quantity of bromine in the urine, add 20-30 cc of pure chloroform and 3 per cent potassium permanganate solution drop by drop until the color is no longer discharged. Finally add 15-20 drops more of the latter solution and allow the separating funnel, stoppered and protected from light, to stand for 15 minutes at rest. Then shake well and, as soon as the chloroform solution of bromine has settled, run as completely as possible into a dry glass-stoppered graduate.

¹ A description of this colorimeter and more detailed information as to its use will be found in Chapter V of this book (see page 468). The comparison-wedges required for bromine determinations may be procured from the Firm of C. Hellge and Co., Freiburg i. B.

Using the same volume of chloroform, shake again, and if much bromine is present even a third portion, and run the extracts into the graduate. Finally dilute the combined extracts with chloroform to a definite volume. Should the chloroform solution of bromine not be entirely clear, shake with pieces of filter paper, or pour quickly through dry paper. With this clear solution, poured into the small glass trough of the colorimeter, determine the color strength as described below for calibration of the comparison-wedge. The quantity of potassium bromide, corresponding to the color strength of the chloroform solution of bromine examined, is determined from the calibration curve of the comparison-wedge by looking on the abscissa for the point in the curve corresponding to the same strength of color indicated by the reading on the scale of the colorimeter. This gives the mg of potassium bromide corresponding to bromine in each 10 cc of chloroform solution of bromine. To calculate the quantity of sodium bromide, multiply the quantities of potassium bromide derived from the curve by 0.8647. $\text{NaBr KBr} = 102.92.119.02 = 0.8647$. Organs of the body, such as liver, or brain, as well as blood, should be finely divided, well-mixed in a nickel crucible with sodium hydroxide prepared from metallic sodium and triturated. Carbonize this material and finally ash it completely by means of potassium nitrate as described above.

Example of Calculation.—24 hour urine 3300 cc. Volume for bromine determination 100 cc. Volume of chloroform solution of bromine 40 cc. Identical color strength scale reading 58 = 7.9 mg of KBr, corresponding to bromine in 10 cc of chloroform solution of bromine. So 40 cc of the latter solution, or 100 cc of urine, contain $4 \times 7.9 = 31.6$ mg and the 24 hour urine $33 \times 31.6 = 1042.8$ mg = 1.0428 grams of KBr. This quantity of KBr corresponds to $1.0428 \times 0.8647 = 0.902$ gram of NaBr.

Notes—Larger volumes of urine, 200 cc and more, should first be evaporated to smaller volume in a porcelain dish over free fire. Evaporation to dryness and ashing should be carried out in a nickel crucible. The mixture of bromide, acid potassium sulphate and potassium permanganate should stand at least 15 minutes, otherwise the results are liable to be too low. Addition of chloroform to this mixture before extracting bromine gradually set free is advisable, otherwise opening the separating funnel and pouring in chloroform may result in loss of bromine from evaporation.

Calibration of Comparison-wedge.—To determine bromine colorimetrically by the Autenrieth-Königsberger colorimeter, fill a glass-stoppered wedge with a chloroform solution of bromine of such dilution that the lower part of the wedge still has a perceptible yellow color. This is the comparison-wedge and is calibrated with potassium bromide solution of known strength. This is done by introducing different but exactly measured volumes of 1:1000 potassium bromide solution into a rather small separating funnel. Each time add 5 cc of dilute sulphuric acid (1:10) and enough water to have the same volume in all cases, for example, 30 cc. Finally add to each portion 10 cc. of pure chloroform and 12–15 drops of saturated potassium permanganate solution. Stopper the separating funnel and allow the mixture to stand protected from light for 10 minutes. Then shake well and pour a portion of the clear chloroform solution of bromine into the small glass trough of the colorimeter. By adjusting the comparison-wedge, determine on the scale where the color strength of the two

solutions is the same. If the chloroform solution of bromine is not clear, shake with filter paper or pour through a small dry filter.

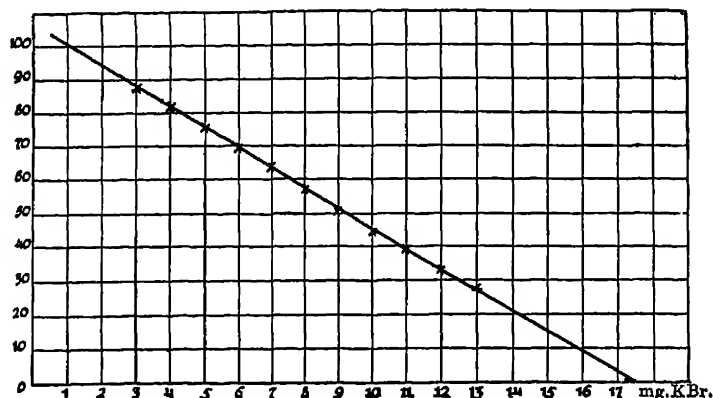


FIG. 23.—Calibration curve of comparison-wedge for colorimetric determination of bromine in bromides. Ten cc of Br-CHCl_3 solution for each reading.

By the calibration of the comparison-wedge in this manner the following values were obtained:

Solution in Cc.	KBr in Mg	Scale-reading
4	4	81-82
5	5	76-77
7	7	63-64
9	9	50-51
10	10	44-45
11	11	40-41
12	12	33-34
13	13	28-29

The calibration-curve (Fig. 23) of the comparison-wedge is obtained by plotting each time the quantity of potassium bromide in mg. upon the abscissae and the reading on the colorimeter scale for equal color strength upon the ordinates.

Iodine and Iodides

Early large quantities of free iodine, for example, tincture of iodine, taken internally, give rise to pain in the mouth, oesophagus and stomach. Vomiting of dark yellow masses, or blue in case the food in the stomach contains starch, may occur as well as diarrhoea, bloody stools and collapse. In rare cases poisoning terminates fatally. A laborer who had drunk 58 grams of tincture of iodine = 5.8 grams of iodine collapsed on the second day and died after 33 hours (R. Kobert). Frequently skin eruptions and diarrhoea result from painting the skin with tincture of iodine. Phenomena that seem to indicate severe potassium iodide poisoning are possible, especially if the mucous surfaces of the throat or the female genitals are painted with tincture of iodine or with Lugol's solu-

tion Robert places the smallest lethal dose of free iodine at 3 grams. When fatal doses of iodine have been taken, death may ensue in 1-2 days, or even later.

In contact with tissues and fluids, iodine disappears, for it combines both with alkalies and protein substances. Alkalinity of blood-serum is thereby lowered. With iodine living protoplasm forms various protein compounds some of which are stable. Red blood-corpuscles are haemolyzed and excess of iodine carries decomposition of dissolved haemoglobin down to haematin. Free iodine kills living cells of organs, for example, those of the liver or kidneys.

After use of potassium iodide, iodism temporarily appears, manifesting itself by metallic taste, flow of saliva, coryza, dizziness, red and watery eyes. Especially skin eruptions (iodine acne) appear, behaving much like bromine acne. Iodine is present in the contents of the pustules.

Iodine is rapidly eliminated from the blood through the urine after use of potassium iodide. Experiments of the author, where 4 grams of this salt were taken, showed that 73 per cent was recovered in the urine after 2 days. The thyroid gland retains part of the iodine. Although alkaline iodides are very quickly eliminated from the blood, even after 8 days, Terrle found appreciable quantities of iodine in the testicles which like the thyroid gland appear to be receptacles of this halogen.

Detection of Free Iodine.—Poisoning from free iodine is often recognizable from brown spots upon the skin or upon wearing apparel. These cannot be mistaken for nitric acid spots, for the latter unlike iodine spots are usually pure yellow and do not gradually disappear with ammonia, potassium hydroxide, or sodium thiosulphate solutions but become dark orange or at least remain unchanged. Because of slight affinity for hydrogen, free iodine persists longer than other halogens and may be recognized by color, odor and violet-colored chloroform solution. Gadamer recommends passing a current of air through the gently warmed material and then through chloroform (violet color), or through starch solution (blue color). Moreover in case of severe, particularly fatal poisoning from free iodine, as well as from tincture, it is to be expected that alcoholic extracts of the material will always contain iodine in inorganic combination, that is, ionic iodine.

Detection and Estimation of Iodine in Urine, Blood and Organs after Administration of Alkali Iodides

Usually a qualitative test for ionic iodine in urine may be made direct. Acidify 20-100 cc of urine with dilute sulphuric acid, add sodium or potassium nitrite solution, and then shake with chloroform. If the latter has a violet color, presence of iodine is shown.

The colorimetric method is more convenient when a quantitative estimation of iodine in organs and fluids of the body must be made. As stated above, alkali iodides taken internally are eliminated rather quickly from the blood through the urine. Usually therefore only small quantities of iodine are found in blood and organs of the body. When it is a question of estimating very small quantities of any substance, colorimetric methods should receive first consideration. Autenrieth and Funk¹ adopted the following procedure with the Autenrieth-

¹ W. Autenrieth and A. Funk: Estimation of Iodine in Urine and Organs. *Münchener mediz. Wochenschrift* 1912, Nos. 49 and 50.

Königsberger wedge-colorimeter A comparison-wedge is first prepared by diluting a chloroform solution of iodine with chloroform until the thin end of the wedge when full shows a perceptible color This comparison-wedge should be kept in the dark

Calibration of Comparison-wedge.—This wedge, filled and kept well closed, serves as a comparison-wedge in the colorimetric estimation of iodine First it must be calibrated In a tared weighing-glass tightly closed with a glass stopper weigh accurately 0.05 gram, or any other aliquot quantity, of purest, dry resublimed iodine Dissolve the iodine in chloroform to a volume of 200 cc. By means of a capillary pipette or burette accurately measure varying volumes of this chloroform solution of iodine, that is, 1, 2, 3 cc, diluting in each case with chloroform to 10 cc in a small graduate In each case pour a test portion of the chloroform solution of iodine of known strength into the small glass-stoppered trough of the colorimeter Adjust the glass wedge and determine the reading where the color of the solution in the wedge has the same intensity as that of the

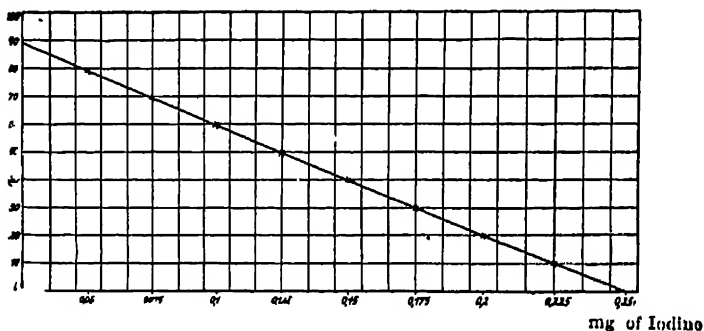


FIG 24—Iodine curve In 1 cc of chloroform solution.

solution in the trough For accuracy it is advisable to repeat this 5-6 times and take the average reading To construct the calibration-curve, take millimeter paper and mark upon the abscissae the quantity of iodine contained in each case in 1 cc of chloroform solutions examined and upon the ordinates the reading on the colorimeter scale for equal color strength The line connecting the points thus recorded is the calibration-curve of the comparison-wedge = Iodine Curve Fig 24

In the calibration of the comparison-wedge by means of a chloroform solution, containing 0.25 mg of iodine per cc., the following intersection points leading to the construction of Fig 24 above were determined for equal color strength:

Chloroform Solution of Iodine		Pure Chloro- form	Iodine in 1 Cc. of Solution	Scale Reading for Equal Color Strength
1 cc	+	9 cc.	0.025 mg	89
2 cc	+	8 cc	0.050 mg.	79
4 cc	+	6 cc	0.10 mg.	58
6 cc.	+	4 cc	0.15 mg.	38
8 cc	+	2 cc.	0.20 mg.	19
10 cc	+	0 cc.	0.25 mg	0

So this comparison-wedge has a range from 0.025 to 0.25 mg of iodine dissolved in each case in 1 cc of chloroform

Estimation of Iodine in Thyroid Gland

E. Baumann's procedure offers a convenient method of carrying out this determination. To get a true average sample, finely comminute the entire thyroid gland, dry at 100° to constant weight, and grind as finely as possible in a small mill. Carefully evaporate 1 gram of this powdered thyroid gland in a clean nickel crucible with about 1 gram of sodium hydroxide prepared from metallic sodium and 5 cc of water. Completely ash carbon particles and any organic matter still present by sprinkling a little powdered potassium nitrate over the mass and fusing to a white melt. Heating should not be too intense nor too prolonged, otherwise traces of alkali iodide may volatilize. Use a rather deep nickel crucible and keep covered. Dissolve the nearly cold melt in hot water, filter the solution and rinse the crucible into a small separating funnel. According to the quantity of iodine present, add 5, 10, 20 or more cc of chloroform and acidify with dilute sulphuric acid, shaking carefully, cooling and allowing liberated carbon dioxide to escape. As soon as most of the latter has passed off, shake well to bring about complete solution of iodine in chloroform. Run the clear chloroform solution of iodine through a dry filter into a graduate. Make two more extractions of the solution in the separating funnel with a little chloroform, passing these extracts through the same filter into the graduate. Mix the combined extracts and determine the volume of the chloroform solution of iodine. Pour a test portion of this solution into the glass-stoppered trough of the colorimeter and by means of the calibrated comparison-wedge determine the point at which its color intensity is the same as that of the solution in the trough. Take the reading on the colorimeter scale and ascertain on the iodine curve the value corresponding to the reading. This gives the quantity of iodine in mg in 1 cc. of chloroform solution. Should the color of the chloroform solution of iodine be too intense, dilute further with chloroform, note the new volume and take another reading.

Estimation of Iodine in Urine

A direct quantitative determination of iodine in urine in the manner described above for the qualitative detection is possible, when urine contains only inorganic combined, that is, ionic iodine, as it does after alkali iodides have been taken internally, provided the acidified urine imparts no color to chloroform. Obviously the volume of the chloroform solution of iodine must be carefully measured. If a direct determination is not possible, evaporate 10 cc or more of urine to dryness with 1-2 grams of sodium hydroxide prepared from metallic sodium in a clean nickel crucible of suitable size. Completely remove carbon particles and any organic matter by sprinkling 1-2 grams of powdered potassium nitrate over the residue and fusing to a white melt. Heating should not be too intense nor too prolonged. In other respects treatment of the filtered aqueous solution of the melt is identical with that described for determination of iodine in thyroid gland.

If pure sodium hydroxide is not available, evaporate the measured volume of urine with 1 gram of pure sodium carbonate and 2-3 grams of potassium nitrate. In this case fusion of the residue should be conducted at gentle heat over an ordinary Bunsen flame until no carbon particles remain and the melt is white.

To express iodine in terms of potassium or sodium iodide, multiply the value obtained for free iodine by 1.309 for potassium iodide $KI \text{ I} = 166.02 : 126.92 = 1.309$, or by 1.19 for sodium iodide $NaI \text{ I} = 149.92 : 126.92 = 1.19$

Note.—Since commercial sodium hydroxide, fused caustic soda, often contains traces of sodium iodide, sodium hydroxide prepared from metallic sodium should be used in the above experiments

Hydrogen Sulphide

Hydrogen sulphide, H_2S , is one of the most toxic substances known. A person inhaling air containing several per cent of hydrogen sulphide drops within a few seconds, becomes unconscious, and dies almost immediately without first having convulsions. This is the so-called apoplectic form of hydrogen sulphide poisoning. Most persons can tolerate only 0.01 per cent of hydrogen sulphide in air. In three cases Lehmann¹ observed that very painful irritation of eyes, nose and throat appeared within 5–8 minutes in an atmosphere containing 0.02 per cent of hydrogen sulphide, and after 30 minutes their condition was hardly endurable. Air containing 0.05 per cent of hydrogen sulphide is as much as a man can stand, for after 30 minutes in addition to pain in the eyes it gives rise to nasal catarrh, difficulty in breathing and coughing, also palpitation of the heart, dizziness, unsteady gait, trembling of the extremities, extreme languor, pallor, cold sweat, headache and unconsciousness. Lehmann states that a man in air containing 0.07–0.08 per cent of hydrogen sulphide will become dangerously ill after several hours and in 0.1–0.15 per cent will die rather quickly. In some cases symptoms of hydrogen sulphide poisoning do not develop until later, after persons apparently in good health have left the polluted atmosphere. Signs of chronic poisoning in the case of workmen exposed almost daily to hydrogen sulphide appear in the form of conjunctivitis (inflammation of the conjunctiva), headache and gastric disturbances. The complexion is pallid and the skin shows a tendency to furunculosis. Habituation to the poison appears not to take place. On the contrary, apparently the sensitiveness of a person to hydrogen sulphide increases. The findings of the autopsy vary. In case of fatal poisoning terminating rapidly they may be entirely negative. When death comes on slowly, a characteristic odor is noticeable in the body-cavities and sometimes in the organs. In case of an immediate autopsy a greenish change of color of the entire cadaver, or at least of the brain or blood, is not noticed but appears some time after burial and then is very characteristic. Every normal cadaver, however, shows this coloration as a result of normal putrefaction but it is longer in appearing!

Detection in Parts of the Cadaver

Only when parts of the cadaver are quite fresh, is a chemical detection of hydrogen sulphide poisoning that is free from objection possible, for this gas is always formed when putrefaction sets in. Suspend in the containers over the parts of the cadaver a strip of paper moistened with lead acetate solution, or pass a stream of carbon dioxide washed with water through the objects tested and note whether the gas that issues colors "lead paper" brownish black as hydrogen sulphide does.

¹ K. B. Lehmann. *Archiv. für Hygiene* 14 (1892), 135.

A spectroscopic examination of the blood of a person poisoned by hydrogen sulphide is often of value. For this purpose examine in the spectroscope a 2.5-3 per cent aqueous solution of blood taken from the brain (Harnack). If the blood contains hydrogen sulphide, a rather sharply outlined absorption-band between C and D appears together with the broad band of reduced haemoglobin. In case of doubt, saturate the given blood with hydrogen sulphide washed through water, make a solution of the above strength, and compare it with the blood in question. If a blood examination immediately follows death from hydrogen sulphide, or from inhalation of air containing less of the gas, the result of this test is usually negative. According to the thickness of the layer, blood containing hydrogen sulphide has a dirty green to dirty brown color.

Detection in Urine

To arrive at a definite conclusion from a urine examination as to poisoning by hydrogen sulphide, the urine should be drawn as freshly as possible, for hydrogen sulphide is set free as a result of putrefaction of certain organic sulphur compounds in every normal urine, such as oxyproteic acid, alloxypoteic acid and cystine. Wash a current of air by means of potassium hydroxide solution, pass it through the urine, and then through a narrow glass tube, suspending at the exit a strip of moist "lead paper." The urine may also be examined by the Caro-Fischer methylene blue test (see below under detection of hydrogen sulphide in water). For this purpose add to the urine 5 drops of hydrochloric acid, 1-2 drops of ferric chloride solution and a few particles of para-amino-dimethylaniline hydrochloride. A blue coloration of the urine mixture shows hydrogen sulphide.

Detection in Air

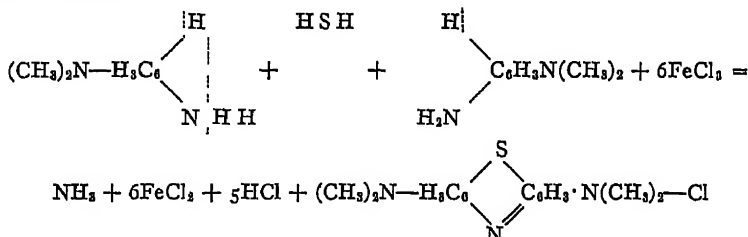
More than traces of hydrogen sulphide may be recognized by its characteristic odor. Also silver and copper objects turn brown or black in an atmosphere containing this gas. "Lead paper" behaves in the same way. A strip of paper saturated with a freshly prepared solution of sodium nitroprusside containing a little ammonia turns violet in presence of hydrogen sulphide. The latter test may also be made by passing the suspected air through dilute ammonia solution containing a few drops of dilute sodium nitroprusside solution. A violet coloration shows hydrogen sulphide. Another good test consists in passing the air through a pure aqueous solution of arsenious oxide (As_2O_3). Hydrogen sulphide colors this intensely yellow from formation of colloidal arsenic sulphide. The latter upon addition of a few drops of hydrochloric acid is precipitated as yellow arsenious sulphide (As_2S_3). The hydrosol of arsenious sulphide is converted into its gel.

Detection in Water

In case presence of hydrogen sulphide in the water cannot be recognized by odor, test with alkaline lead solution (lead acetate solution containing excess of sodium hydroxide solution). A brown coloration, or brownish black precipitate, shows that it is present. A violet coloration in dilute ammonia solution containing a few drops of aqueous sodium nitroprusside solution (1:100) is another

test that may be used The Caro-Fischer reagent¹ is very sensitive to hydrogen sulphide To detect very small quantities of this gas in aqueous solution, add first about $\frac{1}{50}$ of the volume of the solution of fuming hydrochloric acid The drop in a few small particles of para-amino-dimethylaniline sulphate As soon as these have dissolved, add 1-2 drops of ferric chloride solution In presence of hydrogen sulphide the mixture after some time takes on a pure blue color

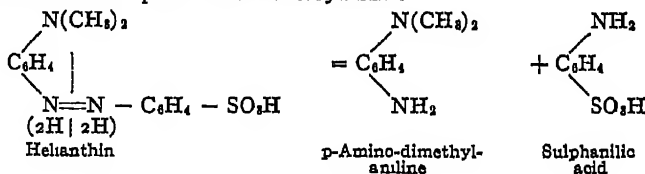
This test depends upon formation of methylene blue when an acid solution of para-amino-dimethylaniline is treated with hydrogen sulphide and a little ferric chloride.



A large excess of hydrochloric acid is used to prevent formation of a red dyestuff when ferric chloride acts upon para-amino-dimethylaniline alone in neutral or faintly acid solution Formation of methylene blue, according to E. Fischer is the most sensitive and positive test for hydrogen sulphide in neutral or acid aqueous solution as the following experiment shows

In this test 1 liter of distilled water containing 0.09 mg. of hydrogen sulphide was taken First 20 cc. of fuming hydrochloric acid (sp. gr. 1.19) were added then a few particles (about 5 mg.) of para-amino-dimethylaniline sulphate, and finally 2 drops of dilute ferric chloride solution The mixture was at room temperature Formation of dyestuff began at once and after about $\frac{1}{2}$ hour reached a maximum The solution had a strong pure blue color that held for a day

Preparation of Amino-dimethylaniline.—According to E. Fischer, the indicator helianthin, or Orange III, para-dimethyl-amino-azo-benzene sulphonic acid, is convenient starting-point in the preparation of this reagent Like most azo dyestuffs it is reduced by nascent hydrogen and is thereby broken up into sulphanilic acid and para-amino-dimethylaniline



Reduction takes place upon heating with hydrochloric acid and zinc dust, or even more easily with ammonium sulphide. Pour about 5 parts of water and excess of ammonium sulphide, that is, 2-4 parts upon finely ground helianthin and shake the cold mixture frequently. The dyestuff dissolves completely

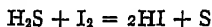
¹E. Fischer Formation of Methylene Blue as a Test for Hydrogen Sulphide Ber. d. Deutsch. chem. Ges. 16 (1883), 2234.

After 24 hours the orange color has disappeared and reduction is complete. If the above mixture is warmed for 10-15 minutes upon the water-bath, cleavage of helianthin takes place more rapidly and smoothly. After complete reduction, extract the solution of para-amino-dimethylaniline with ether. The ether extract also contains some ammonium sulphide which should be removed by agitation with a little lead white suspended in water. Then cautiously add to the ether solution an ether solution of concentrated sulphuric acid. Neutral sulphate of para-amino-dimethylaniline separates as a nearly colorless thick mass. Excess of sulphuric acid should be avoided, otherwise the badly crystallizing acid salt is formed. The precipitated salt separated from ether is warmed upon the water-bath with 4-5 parts of absolute alcohol until it has changed into fine white needles. These are filtered off when cool, washed with alcohol, pressed and dried at 100°. In a closed vessel this preparation remains practically unchanged. Its solution in water has a faint brown color and is well suited for the hydrogen sulphide test.

Quantitative Estimation of Hydrogen Sulphide in Air

(Lehmann)

This method is based upon the reaction taking place between iodine and hydrogen sulphide in aqueous solution:



By means of an aspirator, draw 8-10 liters of air in about 40 minutes through 10 cc. of 0.01 N-iodine solution and then through another flask containing 10 cc. of 0.01 N-thiosulphate solution to catch any iodine carried over mechanically. At the end of the experiment mix the two 0.01 N-solutions, add starch solution as indicator, and titrate excess of thiosulphate solution with 0.01 N-iodine solution. The number of cc. of 0.01 N-iodine solution required corresponds to the quantity of iodine that reacted, according to the above equation, with hydrogen sulphide. 1000 cc. of 0.01 N-iodine solution correspond to $\frac{\text{H}_2\text{S}}{200} = \frac{34}{200} = 0.17$ gram of hydrogen sulphide and 1 cc. of 0.01 N-iodine solution to 0.0017 gram or 0.17 mg. of hydrogen sulphide.

Sulphur Dioxide and Sulphurous Acid

Inhalation of sulphur dioxide produces effects both local and remote, as its aqueous solution does when taken internally. Because of easy conversion into sulphuric acid, like that acid it has a strong local corrosive action. Remote action is due to acidification and decomposition of the blood. These effects are also produced by acid sulphites, whereas local action of neutral salts is milder and less irritating, the latter having rather a paralyzing action upon the nervous system (R. Kobert). Lehmann¹ found that inhalation of air containing in 1000 volumes 0.006-0.010 volumes of sulphur dioxide causes nasal and bronchial irritation. It was unpleasant, though endurable for a short time, to remain in air containing 0.015-0.02 volumes, whereas 0.03 volumes rendered many persons, especially those who were sensitive, quite ill. There was stinging pain in the

¹ K. B. Lehmann: *Archiv. für Hygiene* 18 (1893), 180.

nose, sneezing and coughing. Kobert observed in a ligno-sulphite inhalatorium that individuals differed widely in sensitiveness to inhaled sulphur dioxide, habitation frequently taking place very quickly. Aqueous sulphurous acid and its salts may to some extent give rise to severe toxic effects. The gastric juice sets sulphur dioxide free from sulphites and a 0.5-1 per cent solution of this gas may exert quite a poisonous action, manifesting itself in corrosions, blood decomposition, as shown by coagulation and formation of haematin with brown coloration, and finally paralysis of the nervous system.

Sulphurous acid or its salts should not be used to preserve foods, since even a small quantity they may cause injury to health. In experiments with neutral sodium sulphite, used as a preserving salt, Kionkas added only 0.2 per cent to meat and produced severe blood decomposition in dogs by long continued feeding. Frequently beer and wine contain traces of sulphurous acid, since it is formed in many fermentations. In case of beer it may come from sulphure hops. In a great many wines Kerp¹ determined free sulphurous acid, in 7 per cent of which he detected 1-10 mg per liter and in 5 per cent 11-20 mg. So it may be said that commercial wines in general do not contain more than 2 mg of free sulphur dioxide in the liter. Kerp also detected in wine the presence of acetaldehyde sulphurous acid. In aqueous solution this acid undergoes hydrolytic cleavage into acetic aldehyde and sulphurous acid, depending upon concentration and temperature of solution. Dissociated sulphur dioxide can be titrated directly with iodine solution and is designated as "free sulphurous acid" expressed as SO_2 . Hydrolytic cleavage of acetaldehyde-sulphurous acid is only slight even when the solution is highly diluted. For this reason free sulphurous acid is present in urine only in small quantity.

Detection and Estimation of Sulphurous Acid

(a) Add sulphur-free zinc and hydrochloric acid to the material and by means of lead paper test hydrogen for hydrogen sulphide. A positive test establishes the presence of sulphurous acid. Zinc and hydrochloric acid should be tested alone to make sure that hydrogen sulphide does not come from reagents.

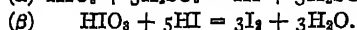
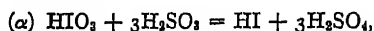
(b) Put in a capacious glass flask, connected on one side with a carbon dioxide generator and on the other side with a Liebig condenser, 200-300 cc. of wine together with 20 cc. of official phosphoric acid (containing 25 per cent. H_3PO_4), and about 10 cc. of 0.1 N-iodine solution in the receiver. Pass a stream of carbon dioxide washed by water through the wine and after about 15 minutes begin distillation. Distil off about one-third of the wine. The distillate should contain excess of free iodine, otherwise more iodine solution must be added. Acidify with dilute hydrochloric acid, heat and precipitate sulphuric acid by barium chloride in the usual manner. The weight of barium sulphate multiplied by 0.274 gives the quantity of sulphur dioxide: $\text{BaSO}_4 (233) : \text{SO}_2 (64) = 1 \times x = 0.274$.

Detection of Sulphur Dioxide in Air

The sharp smell of sulphur dioxide makes possible its detection in air when more than traces are present. Even traces of this gas may be detected in a

¹ W. Kerp. Sulphurous Acid in Wine. *Arbeiten aus dem Kaiserl. Gesundheitsamt* 21 (1904), 141, 156, 180, 372

with certainty by means of paper saturated with starch paste containing iodine acid. Liberation of iodine causes it to turn blue



The suspected air may also be passed through pure water. If sulphurous acid is present, the water will then bleach blue litmus paper and, after addition of chlorine water, give a precipitate with barium chloride showing presence of sulphuric acid.

Detection of Sulphurous Acid as Such in the Cadaver

After fatal poisoning by sulphurous acid, or sulphite, it is hardly ever possible to detect sulphurous acid in the cadaver because of the ease with which it is oxidized. Sometimes an abnormally high percentage of sulphate in stomach contents and blood makes possible the conclusion that poisoning by sulphur dioxide has taken place. The blood also shows change of color. In case of animals poisoned by free sulphurous acid arterial blood has a strikingly dark color and in some instances is said even to react acid (Ogata). On the other hand, neutral sulphites cause blood to take on a brick-red color.

Official Directions (German) for Detection and Estimation of Sulphurous Acid and Salts and of Hyposulphites in Meat

Mix upon the bottom of a 100 cc. Erlenmeyer flask as quickly as possible 30 grams of finely comminuted meat and 5 cc. of 25 per cent. phosphoric acid. Then at once stopper the flask. The end of the cork projecting into the flask has a slit holding a strip of potassium iodate-starch paper moistened for about 1 cm. on its lower end so that it hangs about 1 cm. above the centre of the mass of meat. The solution for this test-paper is made by dissolving 0.1 gram of potassiumiodate (KIO_3) and 1 gram of soluble starch in 100 cc. of water.

If within 10 minutes the strip shows no blue color, which usually appears at the juncture of the moist and dry paper, loosen the cork slightly and set the flask upon the water-bath. If within 10 minutes the strip fails to show either a fugitive or a permanent blue color, then again stopper the flask tightly and allow it to cool in air. Finally, if within 30 minutes the strip shows no blue color, the meat is regarded as free from sulphurous acid. On the other hand, if the strip turns blue, a final test for sulphurous acid should be made by the following method.

(a) Mix 30 grams of finely comminuted meat in a 500 cc. distilling flask with 200 cc. of boiled water, adding sodium carbonate solution until faintly alkaline. Allow to stand 1 hour and then close the flask with a two-holed stopper through which two glass tubes enter the flask. The first tube extends to the bottom of the flask and the second only into the neck. The latter connects with a Liebig condenser having at the other end an air-tight connection by means of a bored stopper with a so-called Péligré tube.

Pass carbon dioxide (water-washed) through the tube leading to the bottom of the flask until air is completely expelled from the apparatus. Then put 50 cc. of iodine solution into the Péligré tube, made by dissolving 5 grams of pure

iodine and 7.5 grams of potassium iodide in a liter of water. This solution should be free from sulphate. Raise the stopper of the distilling flask and, without interrupting the stream of carbon dioxide, run in 10 cc of 25 per cent aqueous solution of phosphoric acid. At once stopper the flask, carefully heat the contents, and distil off half the aqueous solution all the while keeping up the stream of carbon dioxide. Now transfer the iodine solution, which should have a brown color, to a beaker, thoroughly rinse the Pélégot tube with water, add some hydrochloric acid, heat for a short time, and precipitate with barium chloride solution (1 part of crystallized $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ in 10 parts of distilled water) sulphuric acid formed by oxidation of sulphurous acid. Usually it is not absolutely necessary to weigh barium sulphate. If, however, for a special reason the precipitate should be weighed, allow it to settle and make sure that all sulphuric acid is precipitated by adding a drop of barium chloride solution on top of the clear liquid. Again heat to boiling, keep warm and allow to stand for 6 hours, pour the clear liquid through a paper of known ash-content, repeatedly wash the precipitate in the beaker with hot water, each time allowing the liquid to settle until clear and pouring it through the filter. Finally bring the precipitate upon the filter and wash with hot water until the filtrate no longer shows cloudiness with silver nitrate solution. Then dry filter and precipitate, ash in a weighed platinum crucible and ignite. Moisten the contents of the crucible with a little sulphuric acid, expel the latter, ignite gently, allow to cool in desiccator and weigh.

If this test gives a positive result, the meat is considered as having been treated with sulphurous acid, sulphites, or hyposulphites. If necessary to establish whether sulphurous acid came from hyposulphites, the following method may be employed:

(b) Repeatedly stir and leach in a beaker 50 grams of comminuted meat with 200 cc of water and enough sodium carbonate solution to render faintly alkaline. Press out the meat and filter the extract. Acidify strongly with hydrochloric acid, add 5 grams of pure sodium chloride and heat. Filter off the precipitate obtained and wash until wash-water contains neither sulphurous nor sulphuric acid. Then dissolve the precipitate in 25 cc of 5 per cent sodium hydroxide solution, add 50 cc of saturated bromine water and heat to boiling. Now acidify with hydrochloric acid and filter. The entirely clear filtrate gives in presence of hyposulphites in meat an immediate precipitate of barium sulphate upon addition of barium chloride solution.

Boric Acid

For a long time boric acid was regarded as a substance having no action whatever upon the human organism. The belief was general that even after prolonged use the body suffered no injury. But it is now known that boric acid, however used, more especially after injection of its solutions into body cavities, due to absorption may give rise to severe poisoning that may terminate fatally. When food containing small quantities of boric acid as a preservative has been used for a long time, injurious effects have been observed. Frequent use of milk containing boric acid, for example, has been found to cause illness. Nausea and vomiting followed the ingestion of 8 grams of boric acid within an hour. For several hours, pressure and a feeling of fullness in the stomach persisted. Furthermore external use of boric acid for antiseptic irrigation has been the cause

of injurious effects that have even terminated fatally Lewin thinks that individualism enters in, in so far as it influences elimination of boric acid, making it slow or rapid In general boric acid is eliminated slowly by way of the saliva and urine Any interference, however slight, with elimination of boric acid has brought about its accumulation in the body and given rise to unpleasant effects Schlenker¹ has shown from metabolism experiments on man that daily use of 1.5-3 grams of boric acid has a strong influence upon the absorptive power of the intestinal tract and causes excretion of considerable intestinal mucus. Many investigators who have studied the question of harm done to the human body by boric acid and borax have concluded from their experiments that even small quantities of these two substances may cause irritation of the gastro-intestinal tract This may manifest itself in vomiting, diarrhoea, impaired digestion of food and consequent emaciation Skin eruptions may also appear, as eczema, petechiae,² erythema and oedema Boric acid is deposited in the organs, that is, a part of the acid is stored up and slowly eliminated by the salivary glands, gastric mucosa, milk glands, but mainly by the blood through the kidneys. Nephritis is said to arise possibly from this cause Disturbances of vision have also been observed (Kobert)

As the result of experiments in which borax was fed to dogs, boric acid was detected in the liver, bile, kidneys, blood and lungs After subcutaneous injection it was found also in the gastro-intestinal mucosa.

Detection of Boric Acid

Flame Test.—In examining urine, render it strongly alkaline with sodium carbonate, evaporate to dryness upon the water-bath, mix the residue well with concentrated sulphuric acid (4-5 cc.), add alcohol (preferably methyl alcohol), stir again and ignite the alcohol. In presence of boric acid, the yellow flame will have a green mantle which may be seen best by stirring the mixture with a glass rod. The green color of the flame may also be seen to advantage in a dark room.

Turmeric Test.—Evaporate the urine, or comminuted organ, to dryness with sodium carbonate and potassium nitrate. Ignite the residue but not too strongly nor too long. Dip a strip of turmeric paper in the solution of the residue after acidification with hydrochloric acid. In presence of boric acid the paper has a red-brown color which comes out especially when the paper is dried. Then if moistened with potassium hydroxide solution the color of the paper changes to dark greenish blue or blackish blue.

¹ G. H. Schlenker: Use of Boric Acid as a Food-Preservative Dissertation, München 1883.

² Petechiae are small, usually roundish hemorrhages about the size of a pin-head in the corium They differ from erythema in not losing their color upon pressure

Quantitative Estimation of Boric Acid

1. Jørgensen's¹ Method.—Boric acid in aqueous solution titrated with sodium hydroxide solution and phenolphthalein gives a red color after addition of a slight amount of alkali, approximately 1 mol. of sodium hydroxide to 6 mols. of boric acid. In presence of considerable glycerol, or mannitol, boric acid behaves like a mono-basic acid, that is, a solution of 1 mol. of boric acid with phenolphthalein turns red after addition of exactly 1 mol. of sodium hydroxide solution. For example, dissolve 0.4–0.5 gram of boric acid in as little water as possible, add 50 grams of neutral glycerol and phenolphthalein solution, and titrate with 10 or 0.1 n-sodium hydroxide solution to red color. The equivalent weight of boric acid = mol. wt. = H_3BO_3 = 61. So 1 cc. of 0.1 n-sodium hydroxide solution under the above conditions neutralizes 0.061 gram of boric acid.

Application of Method to Estimation of Boric Acid in Meat, Milk, Urine, Etc.—Add sodium hydroxide solution to 50 grams of meat, 100 cc. of milk or urine, until strongly alkaline. Mix thoroughly, dry, and ash. Dissolve the residue in dilute sulphuric acid, warming to expel carbon dioxide. Cool, dilute to 50 cc., add phenolphthalein and, disregarding any precipitate (H_3BO_3 ?), exactly neutralize with 0.1 n-sodium hydroxide solution. Then add 50 cc. of neutral glycerol and titrate to the end with 0.1 n-sodium hydroxide solution. Under as nearly as possible the same experimental conditions determine the titer of this sodium hydroxide solution, using boric acid solution (5 cc.) of known content, for example, one containing 0.1 gram of pure crystallized boric acid in 50 cc. Calculate the titer on the basis of the number of cc. of 0.1 n-sodium hydroxide solution used after addition of glycerol. Addition of ethyl alcohol at the end of the titration of boric acid is said to make the end-reaction sharper.

2. Partheil and Rose² Method.—Boric acid distills rather easily with steam, also with the vapor of methyl or ethyl alcohol. Commercial ether distilled with boric acid carries over small quantities of the acid. But no boric acid volatilizes when ether is sucked

¹ G. Jørgensen: Volumetric Estimation of Boric Acid. *Zeitschr. f. angew. Chemie* 1897, 5.

² A. Partheil and J. A. Rose. Direct Gravimetric Estimation of Boric Acid. *Ber. d. Deutsch. chem. Ges.* 34 (1901), 3611.

off over boric acid *in vacuo* over sulphuric acid. Boric acid dissolves but slightly in pure absolutely dry ether; it is considerably more soluble in pure ether saturated with water, 100 grams of the latter solvent dissolving 0.188 gram of boric acid. Based upon these observations Partheil and Rose have devised a method of estimating boric acid gravimetrically by means of the extraction apparatus¹ shown in Fig. 25

Procedure.—Pour the boric acid solution acidified with hydrochloric acid into the spirally wound tube until at the highest it reaches only to the bulb. Then carefully add enough freshly rectified ether nearly to fill the bulb. Put 20 cc. of the same ether in the flask weighed with a piece of pumice-stone used to prevent bumping. Then heat upon the water-bath and have the ether in lively ebullition so that it comes from the condenser not in drops but in a continuous stream. In this manner extract for at least 18 hours. Then put on a second weighed flask to make sure after about 2 hours extraction that exhaustion is complete. In each case the flask with the ether solution is put into a vacuum desiccator (Fig. 13, page 96) over sulphuric acid, the ether sucked off, and residual boric acid dried to constant weight and weighed as H_3BO_3 . Using this method, Partheil and Rose obtained 0.3105 and 0.31025 gram of boric acid from an aqueous solution containing 0.31015 gram of the acid. A borax solution gave 0.12425 instead of 0.12408 gram of boric acid.



FIG. 25 —Partheil-Rose extractor.

Notes.—Boric acid to be extracted with ether should not contain free sulphuric phosphoric or nitric acid, or appreciable quantities of iron. The extractor recommended by Partheil and Rose may be used to advantage in extracting alkaloids from aqueous solutions rendered alkaline with sodium hydroxide solution.

This method is applicable to determination of boric acid in margarine, for example. Melt 50 grams of the sample with 20 cc. of water and allow the fat to solidify again completely. Perforate the crust and decant the aqueous solution. Repeat this operation 3–4 times. Render the combined aqueous extracts alkaline with sodium hydroxide solution, evaporate, ash, and extract the solution of the ash in hydrochloric acid as above. Preceding extraction with ether, remove sul-

¹ This extractor is supplied by the Firm of C. Gerhardt, Bonn.

phuric acid with barium chloride, phosphoric acid with ferric chloride, as well as nitric acid, from the alkaline residue left upon evaporation. Directions as follows are given¹ for milk and meat. Ash 50 cc of milk, or 20 grams of chopped meat, with 1 gram of sodium carbonate. Add a few drops of ferric chloride solution to the faintly acidified filtrate of the ash, to remove completely PO_4''' , precipitate excess of iron with sodium hydroxide solution, filter, acidify the filtrate with hydrochloric acid, evaporate to 15 cc, and extract in the extractor with ether. Proceed with the examination of urine in the same manner.

Official Directions (German) for Detection of Boric Acid and Salts in Meats

Make a uniform mixture in a beaker of 50 grams of finely comminuted meat with a mixture of 50 cc of water and 0.2 cc of hydrochloric acid (sp. gr. 1.124 = 25 per cent). Allow the mass to stand in the beaker for half an hour covered with a watch-glass, stirring occasionally, and heat for half an hour in a boiling water-bath. Throw the contents of the beaker while warm upon a piece of cheesecloth, press the meat well, and pour the liquid through a moist filter. Add phenolphthalein to the filtrate, render slightly alkaline with 0.1 N-sodium hydroxide solution, and concentrate to 25 cc. Acidify 5 cc of this liquid with 0.5 cc of hydrochloric acid (sp. gr. 1.124), filter, and test for boric acid with turmeric paper (see Preparation of Reagents, page 644). To do this, moisten a strip of smooth turmeric paper 8 cm. long and 1 cm. wide for about half its length with the acidified solution, drying at 60–70° upon a watch-glass about 10 cm. in diameter. If the turmeric paper shows no visible change in its original yellow color, the meat contains no boric acid. But, on the other hand, if a reddish or orange-red color appears, touch the paper where it has changed color with a 2 per cent. aqueous solution of sodium carbonate. If this produces a red-brown spot not differing in color from the red-brown spot developed by sodium carbonate solution alone upon pure turmeric paper, or a red-violet color, the meat also contains no boric acid. But if the sodium carbonate solution produces a blue spot, presence of boric acid is shown. In case of blue-violet colorations and in doubtful cases, the result of the flame test is conclusive. This should be made in the following manner.

Evaporate to dryness and ash in a platinum dish 5 cc. of the residual alkaline liquid. To prepare the ash, lixiviate the carbonized substance with about 20 cc. of hot water. Completely ash the carbon over a small flame, add the liquid from lixiviation, and bring to dryness first upon the water-bath and then at about 120°. Carefully triturate the light ash thus obtained with a cold mixture of 5 cc. of methyl alcohol and 0.5 cc. of concentrated sulphuric acid, using 5 cc. more methyl alcohol to transfer to a 100 cc. Erlenmeyer flask. Frequently shake the closed flask and allow to stand for half an hour. Then completely distil off methyl alcohol from a water-bath at 80–85°. Transfer the distillate to a glass vessel about 6 cm. high having a volume of 40 cc. Close this by means of a two-hole stopper through which two glass tubes pass, one extending to the bottom the other only as far as the neck. The outer end of the latter tube is reduced in bore and provided with a perforated platinum tip that may be made from foil. Pass through the liquid a stream of dry hydrogen so that the ignited flame is about

¹ A. Partheil and J. Rose: Direct Gravimetric Estimation of Boric Acid in Food-stuffs. *Zeitschr. f. d. Untersuchung von Nahrungs-Genussmitteln* 5 (1902), 1049.

2-3 cm long If the flame observed in diffused daylight has a green color, the meat contains boric acid Meat in which boric acid is detected after carrying out these directions is to be regarded as having been treated with boric acid or its salts.

Chloric Acid and Chlorates

Large doses (4-10 grams) of potassium chlorate, KClO_3 , are decidedly toxic. In the case of adults 15-30 grams of this salt are usually fatal In many instances death does not ensue until several days later. During the first stage of intoxication, alteration in the shape of red corpuscles and conversion of oxyhaemoglobin in the intact corpuscles into brown methaemoglobin take place Then red blood-corpuscles, at least in a case of severe poisoning, change their form, becoming shriveled, and undergoing decomposition Toxicologists (see R Kobert, intoxicationen) ascribe change of blood-pigment and red blood-corpuscles to specific salt action possessed in high degree by potassium chlorate This explanation also accounts for salt diuresis, appearing at the beginning of potassium chlorate poisoning, whereby the blood is much thickened But most notable is the high alkalinity of the urine, resulting in decreased alkalinity of the blood plasma In severe chlorate poisoning so much oxyhaemoglobin is changed to methaemoglobin that the amount of oxygen in the blood may drop to 1 per cent As a result, human beings or animals thus poisoned may become asphyxiated from lack of oxygen Potassium chlorate through the action of potassium weakens the heart In chlorate poisoning the blood has a characteristic chocolate-brown color

Potassium chlorate taken by the mouth is rapidly absorbed from the stomach and soon eliminated by the kidneys After administration of 0.1 gram of potassium chlorate, chloric acid appears in the urine in an hour Most of the potassium chlorate passes into the urine unchanged, only a little of the salt being reduced to potassium chloride During chlorate poisoning, the urine is usually very dark, even black, and may contain haemoglobin and methaemoglobin It is frequently opaque and strongly alkaline Upon long standing a dark brown sediment gradually deposits. The urine also contains considerable albumin

In suspected chlorate poisoning, the urine should if possible receive thorough chemical and microscopical examination An anuria lasting several days may precede death and render an examination of the urine quite impossible

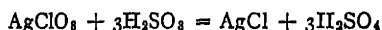
Detection of Chloric Acid

To isolate potassium chlorate from organic material, use a dialyzer that should be as flat as possible, because the thinner the layer in the inner container and the larger the volume of water in the outer vessel, the more rapid the diffusion. Place the material, such as parts of organs and stomach or intestinal contents, in the inner container of a flat dialyzer and pure water in the outer vessel. Allow dialysis to take place 5-6 hours without changing the water in

the outer vessel. Then evaporate the dialysate (contents of the outer vessel) to dryness in a porcelain dish upon the water-bath. Dissolve the residue in a little water and examine the filtered solution for chloric acid (ClO_3^- ion) as follows:

1. **Indigo Test.**—Add dilute sulphuric acid and a few drops of indigo solution, until the color is distinctly blue. Then introduce sulphurous acid drop by drop. If chloric acid is present, the blue color changes to yellow or greenish yellow. This is a delicate test for chloric acid, given even by 0.01 gram of potassium chlorate.

2. **Silver Nitrate Test.**—Add silver nitrate solution in excess. If there is a precipitate (AgCl), filter, add a few drops of sulphurous acid to the clear filtrate, as well as an excess of dilute nitric acid, and boil. A chlorate will cause the precipitation of more silver chloride. Silver chloride differs from silver sulphite in being insoluble in hot dilute nitric acid. Sulphurous acid reduces silver chlorate to chloride.



3. **Free Chlorine Test.**—A solution containing a chlorate, heated with concentrated hydrochloric acid, gives free chlorine. The gas passed into potassium iodide solution liberates iodine. Shake the solution with chloroform which dissolves iodine with violet color. This test indicates chloric acid only in absence of substances like chromic acid and dichromates which also give chlorine with hydrochloric acid.

Quantitative Estimation of Chloric Acid

To estimate potassium chlorate quantitatively in urine, dialysates and other liquids, reduce with zinc dust (I), or employ Scholtz's method (II¹).

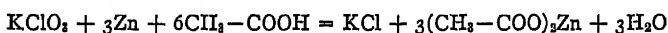
I **Zinc Dust Method.**—Divide the solution into two equal parts. Determine chloride gravimetrically in one portion by precipitating and weighing AgCl , or volumetrically by titrating according to Volhard's method.

In the second portion determine chloride and chlorate together. Add 5–10 grams of zinc dust and a little dilute sulphuric acid, or better acetic acid, and heat the mixture 0.5–1 hour upon a boiling water-bath. Filter and wash the residue with boiling water. Acid-

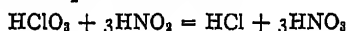
¹ M. Scholtz: Volumetric Estimation of Chlorates and Bromates. *Archiv d. Pharmaz.* 243 (1905), 353

ify the filtrate with dilute nitric acid and determine Cl⁻-ion as above. More chlorine appears in the second than in the first determination. Calculate percentage of potassium chlorate from the difference between the two chlorine determinations. One molecule of KClO₃ upon reduction yields 1 molecule of KCl and therefore 1 molecule of AgCl (143.35) corresponds to 1 molecule of KClO₃ (122.45).

Zinc dust together with acetic acid reduces potassium chlorate to potassium chloride:



II. Method of Scholtz.—This method makes use of the reducing action of nitrous acid upon chloric acid:



Add to the solution, for example, the evaporated dialysate, 10 cc of nitric acid (sp. gr. 1.2 = 32 per cent.) and 10 cc. of 10 per cent. sodium nitrite solution. Allow the mixture to stand for 15 minutes at room temperature. Then add 30–50 cc. of 0.1 n-silver nitrate solution and 5 cc of saturated iron alum solution. Titrate excess of silver with 0.1 n-ammonium sulphocyanate solution. 1000 cc. of 0.1 n-AgNO₃ = 0.1 KClO₃ gram = 12.245 grams of KClO₃.

The slight excess of nitrous acid has no effect upon the delicacy of the reaction. Liquids like dialysates of stomach-contents and organs always contain chloride. In that case first determine the quantity of chloride by means of 0.1 n-silver nitrate solution, using potassium chromate as indicator. Nitrous acid does not reduce an aqueous solution of potassium perchlorate, KClO₄, which may possibly be present.

Hildebrandt¹ has adapted Scholtz's method to the examination of urine. First completely precipitate chloride in a measured volume of urine with silver nitrate in presence of nitric acid. Add sodium nitrite solution to the clear, chloride-free filtrate, as well as more silver nitrate solution, until a precipitate no longer appears. Determine as usual the weight of silver chloride obtained. The corresponding quantity of potassium chlorate is obtained by multiplying this weight by 0.854 : KClO₃ : AgCl = 122.45 : 143.33 = 0.854.

In the case of urine a larger quantity of nitrous acid is decomposed by urea:



Consequently do not use too little sodium nitrite.

¹ H. Hildebrandt. Detection of Chlorates in Urine. Vierteljahrsschr. f. gerichtl. Medizin u. öff. Sanitätswesen 32 (1906), 81.

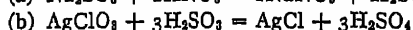
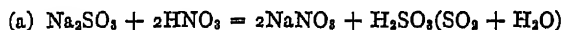
Behavior of Potassium Chlorate in Putrefaction

C Bischoff states that potassium chlorate, mixed with moist, organic substances, especially blood, is very soon reduced to chloride! Bischoff describes several cases, in which poisoning by potassium chlorate had undoubtedly occurred, and yet chloric acid could not be detected chemically in parts of the cadaver. In an experiment, 100 grams of blood, 0.5 gram of potassium chlorate and 100 grams of water were allowed to stand for 5 days at room temperature. Not a trace of chloric acid could be detected in the dialysate. Bischoff concludes from this experiment that potassium chlorate, mixed with moist organic substances, especially with blood, is soon reduced. Consequently, chloric acid may not be detected, even in cases of rapidly fatal poisoning by potassium chlorate.

Official Directions (German) for Detection of Chlorates in Meat

Allow 30 grams of finely comminuted meat to stand for 1 hour in the cold with 100 cc. of water and then heat to boiling. Filter when cold and add silver nitrate solution in excess to the filtrate. Add 1 cc. of 10 per cent sodium sulphite solution and 1 cc. of concentrated nitric acid to 25 cc. of the clear filtrate from the silver precipitate and then heat to boiling. If a precipitate appears, insoluble in more hot water and consisting of silver chloride, chlorate is present. Meat examined according to these directions and giving a positive test is regarded as having been treated with chlorates.

Explanation—Chlorate added to meat as a preservative gives with silver nitrate silver chlorate, AgClO_3 , which is easily soluble in water. Free sulphurous acid formed by the action of nitric acid upon sodium sulphite, reduces silver chlorate to chloride.



To prove conclusively that the precipitate is silver chloride, collect it upon a filter, wash and dissolve in ammonia. Acidification of this solution with Cl^- -free nitric acid will again precipitate silver chloride white and curdy.

Oxalic Acid¹

Oxalic acid and its salts, for example, salt of sorrel, are quite toxic and rapid in action. Administration of oxalic acid has terminated fatally in the case of adults in a few minutes. Oxalic acid is very abundant in the plant kingdom in the form of its acid potassium salt, KHC_2O_4 , and calcium salt. Sorrel, wood-sorrel, and rhubarb are especially rich in salts of oxalic acid. Hence this acid may find access to the body through food and drugs of vegetable origin. Moreover, oxalic acid in small quantity is a normal constituent of human urine, 2-10 mg. being excreted in the course of a day. Con-

¹ For reasons of convenience oxalic acid is included among inorganic substances in connection with inorganic acids.

sequently in examining parts of organs, stomach-contents, urine, and other materials it is often necessary to supplement a positive qualitative test by a quantitative estimation of oxalic acid.

Toxic Action—An important difference between mineral acids and oxalic acid is the toxicity of the salts of the latter. Not only do free oxalic acid and its acid potassium salt, salt of sorrel, show poisonous properties but even very dilute solutions of neutral sodium oxalate, $\text{Na}_2\text{C}_2\text{O}_4$, act in the same way. Therefore in oxalic acid poisoning it is necessary to distinguish between local corrosion, occurring at the point of application and also in part upon elimination, and remote action due to absorption. Local action at the point of application is corrosive like that of all acids. Local action at the place of elimination depends upon formation and insolubility of calcium oxalate. On account of the ease with which the organism takes up oxalic acid and its alkali salts, action of the absorbed poison is rapid. The effects caused by its presence may be ascribed to the fact that this acid removes in part from organs, as the heart, and from body fluids (blood) calcium they require for their life processes, converting it in part into insoluble calcium oxalate. Oxalates diminish coagulating power as well as alkalinity of the blood. On the other hand, they increase the quantity of sugar in the blood. In oxalic acid poisoning there is a depression of the entire metabolism. This is also the case as regards taking up oxygen and giving off carbon dioxide. The body temperature falls as processes of metabolism are retarded. Owing to withdrawal of calcium from the heart, that organ is weakened and finally paralyzed. Local action upon the kidneys is due to clogging of the injured urinary tubules by deposits of calcium oxalate. Flow of urine may wholly cease in consequence of total impairment of the urinary tubules and death may ensue from anuria and uraemia. Fatal poisonings from large doses of oxalic acid are usually of short duration. R. Kobert (Intoxikationen) describes a case where death occurred within 10 minutes.

Bischoff¹ has made statements with regard to distribution of oxalic acid in different organs of persons poisoned by this substance. In a case that terminated fatally in less than 15 minutes the quantity of oxalic acid in each organ was determined separately and found to be

Weight	Organ	Oxalic Acid
2240 grams	Stomach, oesophagus, intestine and contents	2.28 grams
770 grams	Liver	0.285 gram
290 grams	Kidneys	0.0145 gram
180 grams	Blood from the heart	0.0435 gram
40 grams	Urine	0.0076 gram

The quantity of oxalic acid in the liver is noticeably large. The kidneys and urine contain only a little of the poison, owing to the short duration of life after poisoning. A striking thing about the urine excreted during oxalic acid poisoning is the abundant deposition of crystallized calcium oxalate.

¹ C. Bischoff. Distribution of Poisons in the Human Organism in Poisoning Cases. Ber. d. Deutsch. chem. Ges. 16 (1883), 1337.

Detection of Oxalic Acid

To detect oxalate without discriminating between free acid, acid potassium salt, or calcium oxalate, employ the following method:

Add to the finely divided material 3-4 volumes of alcohol and acidify with dilute hydrochloric acid. Stir frequently and allow the mixture to digest 1-2 hours in the cold. Then filter through creased paper moistened with alcohol and wash the residue with alcohol. To prevent formation of ethyl oxalate during evaporation, add about 10 cc. of water to the total filtrate. Evaporate upon the water-bath until all alcohol is expelled. Pass the aqueous residue through a small filter. Extract the filtrate in a separating funnel 3-4 times with 50-60 cc. portions of ether. Allow the total ether

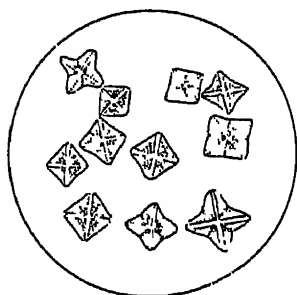


FIG 26 —Calcium oxalate crystals

extract to stand for some time in a dry flask, then pass through a dry filter and distil. Dissolve the residue in 2-3 cc. of water and pass the solution, if necessary, through a moist filter. Add ammonium hydroxide solution until alkaline and then saturated calcium sulphate solution. If a precipitate appears, acidify with acetic acid and allow solution and precipitate to stand over night in a covered beaker. If there is still a crystalline precipitate, it

can be only calcium oxalate. A microscopic examination of this precipitate is advisable. Calcium oxalate forms characteristic octahedrons having the so-called envelope-shape (Fig. 26). But other crystal forms may also appear. When thoroughly washed, calcium oxalate may be converted by ignition into calcium oxide that may be weighed.

Calculation.— $\text{CaO (56):H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O (126) = Wt. CaO found: x}$. Since the quotient $126:56 = 2.25$, multiply the weight of calcium oxide found by 2.25 to get the corresponding amount of crystallized oxalic acid. Or dissolve the washed calcium oxide upon the filter with hot dilute sulphuric acid, warm the solution to about 80° , and titrate oxalic acid with approximately 0.1 N-potassium permanganate solution standardized against pure crystallized oxalic acid: 1000 cc. of 0.1 N- KMnO_4 solution = 6.3 grams of oxalic acid, $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$.

Nitrous Gases and Nitrites

So-called nitrous gases, or vapors, appearing as red fumes from the action of concentrated nitric acid upon many substances, especially those of organic nature, really consist of nitrogen dioxide, NO_2 . Fuming nitric acid also contains this gas. Severe poisonings, some terminating fatally, due to inhalation of too large quantities of these nitrous gases, have been of frequent occurrence in factories making nitric acid, oxalic acid, nitrobenzene and picric acid. Toxic effects manifest themselves in difficult breathing, oppression of the chest, coughing, dizziness, cyanosis and bronchitis. During the early stages of intoxication vomiting of lemon-yellow masses, diarrhoea and collapse may also appear. In many instances the poisoning takes an entirely different course. After having breathed the poisonous vapors, the individual at first has almost no distress and even for an hour can continue working in rooms containing nitrogen dioxide. Then as a rule he experiences a feeling of thirst and need of fresh air. Usually after 6-8 hours without apparent outside cause marked difficulty in breathing and a feeling of uneasiness appear. He has burning thirst and thinks he is going to suffocate. The face is covered with cold sweat and, owing to formation of methaemoglobin, takes on a blue-gray color (cyanosis). The eyes are much swollen. At intervals lasting for several minutes convulsive attacks of coughing attended with vomiting occur. Within 40 hours death ensues from oedema and haemorrhage of the lungs. The first day the urine, always present in small quantity, may contain haematin and methaemoglobin, but not always, and an abundance of albumin.

Traces of nitrites occur normally in the human and mammalian organism, particularly in saliva, nasal mucus, bronchiae, parotis, lung tissue, kidneys, white brain-substance, lymph glands, and testicles (Stepanow¹). This explains why all these animal fluids and organs in presence of carbon dioxide liberate iodine from potassium iodide (catarrh from iodine). It should also be noted that formation of nitrite is possible in the intestinal tract from the action of ever present reducing bacteria upon nitrates in food. Oxidation of ammonia also gives rise to nitrites. There are two classes of nitrite-forming bacteria, one causing formation by reduction of nitrates and the other by oxidation of ammonia (Maassen²).

Nitrites act upon blood-pigment, changing it, that is, oxyhaemoglobin, into methaemoglobin. They also have an irritating action upon mucous membranes, that is, upon those of the stomach and intestines. Finally they cause paralysis of the central nervous system, acting first upon the brain and then upon the spinal cord.

Sodium nitrite, however, seems to be rather mild in its action. Kobert cites the case of a patient who took hourly several doses of 0.5 gram of this salt without serious results. The only effects were nausea, diarrhoea, feeling of faintness, marked cyanosis, and increased diuresis.

¹ A. Stepanow: Detection of Nitrites in Many Organs. *Archiv. f. exper. Path. u. Pharmacol.* 47 (1902), 411.

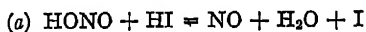
² A. Maassen: Decomposition of Nitrates and Nitrites by Bacteria. *Arbeiten aus d. Kaiserl. Gesundheits-Amte* 18 (1901), 1.

Post-mortem Appearances.—Following inhalation of nitrous gases, the mucous membranes of the larynx, trachea and bronchiae are brownish in color. The blood in the various organs is of a black-brown color and deficient in alkalinity. The exudate upon the surface of an incision in the lungs is brownish.

Detection

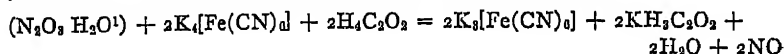
To detect nitrites, dilute the material, such as stomach-contents, or vomitus, with water, acidify with acetic acid, and distil in a current of carbon dioxide, collecting the distillate under water. The following tests may be made.

1. **Starch-iodide Test.**—Add to a portion of the distillate starch-potassium iodide solution acidified with dilute sulphuric acid. Nitrous acid liberates iodine which produces a blue color with starch.



2. **Azo-Dye Test.**—Shake 10 cc of distillate with 10 drops of naphthol reagent (see page 643) and 2 drops of concentrated hydrochloric acid. Then carefully add ammonia as an upper layer. In presence of nitrous acid a red ring appears at the zone of contact. If the tube is turned around, the entire liquid assumes a red color from formation of an oxy-azodyestuff.

3. **Prussiate of Potash Test.**—Even in very high dilution (1 600,000) potassium ferrocyanide solution in presence of acetic acid with nitrous acid turns yellow with oxidation to potassium ferricyanide and nitric oxide.



4. **Bismarck Brown Test.**—Nitrous acid colors an aqueous solution of meta-phenylene diamine (0.5 100) yellow-red to brown (formation of Bismarck Brown). A strip of filter paper moistened with the diamine solution may be used to detect nitrogen dioxide in the air. This test may be made more delicate, if a larger quantity of air is drawn through a solution of the diamine sulphate by an aspirator.

In addition to these tests, nitrous acid also gives the well-known nitric acid tests with ferrous sulphate, brucine and diphenylamine (see page 293). Nitrous acid differs from nitric acid in discharging the color of a solution of potassium permanganate to which sulphuric acid has been added.

Detection of Free Alkalies

Potassium, Sodium and Ammonium Hydroxides

In the detection of alkalies, the same general principles used in detecting mineral acids are applicable. Since potassium and sodium compounds are normal constituents of animal and plant organisms, and since ammonia is a decomposition product of nitrogenous organic matter, examination must always show that alkalies are in the free

¹ Instead of 2HNO_2 is written $(\text{N}_2\text{O}_3, \text{H}_2\text{O})$.

state, for they alone and their carbonic acid salts decompose and corrode animal tissues and not their neutral salts.

Poisonings due to caustic alkalis resemble those of corrosive acids. If taken internally, corrosive action gives rise to pain in the mouth, throat, oesophagus, stomach and abdomen. Mineral acid corrosions are dry and brittle ("solid mortification"), whereas those from caustic alkalis are soft and greasy. The alkali albuminates formed become gelatinous, swell and may partially dissolve in presence of much water. In forensic medicine the term "Colliquation" is used (softening, liquefaction). Destructive action of caustic alkalis extends deep and affects the parts around the corroded places. In caustic alkaline solutions gelatinous tissues, horny substances, hair and skin swell considerably and finally dissolve. The stomach in alkali poisoning is softened, corroded and decidedly bright red in color.

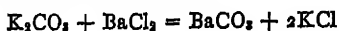
Ammonia

Free ammonia is usually recognized by odor. A piece of moist red litmus paper, held over the material, becomes blue. A paper moistened with mercurous nitrate solution is blackened.

Distillation.—If material is strongly alkaline, extract several times with absolute alcohol. Use a flask with glass stopper and distil the combined extracts. Collect the distillate in a little dilute hydrochloric acid and evaporate the solution to dryness upon the water-bath. Dissolve the residue in water and test the solution for ammonia, using Nessler's reagent, chloroplatinic acid, and sodium cobaltic nitrite, $\text{Na}_3[\text{Co}(\text{NO}_2)_6]$. The residue from distillation may be used in testing for fixed alkalis.

Fixed Alkalies

The residue from distillation may contain potassium and sodium hydroxides. If strongly alkaline, first add a few drops of phenolphthalein solution and then excess of barium chloride solution. The red color and alkaline reaction, if due to carbonates, disappear because two neutral salts are formed:



But if fixed alkalis are present, alkaline reaction and red colors remain, for soluble barium hydroxide is formed:



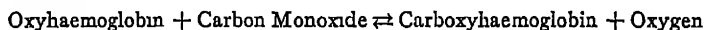
The solution of the latter reddens phenolphthalein.

To distinguish potassium from sodium hydroxide, neutralize the remainder of the residue from distillation of alcohol with dilute hydrochloric acid and test the solution for potassium with chloroplatinic acid, $H_2[PtCl_6]$, and sodium cobaltic nitrite, $Na_3[Co(NO_2)_6]$, and for sodium with potassium pyro-antimonate, $K_2H_2Sb_2O_7$

Carbon Monoxide

I Detection and Estimation in Blood of Carboxyhaemoglobin in Carbon Monoxide Poisoning

Carbon monoxide, CO, is a blood poison. When inhaled, it displaces loosely bound oxygen in oxyhaemoglobin of blood and then combines with reduced haemoglobin forming carboxyhaemoglobin. Haemoglobin of blood thereby loses its power of combining with oxygen and again forming oxyhaemoglobin, the oxygen-carrier of the organism. Hufner states that displacement of oxygen in blood by carbon monoxide takes place in accordance with the laws of chemical mass action. Hence in many poisoning cases the toxic action of carbon monoxide may be overcome by intensive artificial respiration of oxygen:



Death ensues when the haemoglobin-content of the blood-pigment still free from carbon monoxide drops to 35-30 per cent., that is, when 65-70 per cent. of the oxyhaemoglobin has been converted into carboxyhaemoglobin. Consequently blood of a person poisoned by carbon monoxide can never be saturated with carbon monoxide, since death ensues before saturation takes place. It is a notable fact that carbon monoxide blood at first easily gives off carbon monoxide by passing through it a current of air or by pumping. Later it parts with the gas only with great difficulty. Affinity of oxygen for haemoglobin is said to be 210 times less than that of carbon monoxide. This explains why, in respiration of a mixture of carbon monoxide and air, blood can enrich itself with carbon monoxide even when the quantity of poison in air is very small. Gruber found that air containing 0.02 per cent. of carbon monoxide is at the limit of toxicity. When the percentage of carbon monoxide is 0.05 per cent. positive toxic action takes place. Air containing 1 per cent. of carbon monoxide rapidly exerts toxic action. The view that complete saturation of blood does not take place during life is supported by the observations of L. Smith in the case of several

persons who died from poisoning by illuminating gas and whose blood was not over 83 per cent saturated with carbon monoxide. Illuminating gas contains 8-11 per cent. of carbon monoxide and so-called coal gas 0.3-5 per cent. Percentage of carbon monoxide in the latter varies with the nature of the coal from which it is formed.

Blood containing carboxyhaemoglobin may be recognized by its light cherry-red to violet color. The foam in particular formed by shaking has a violet color. In distinction from normal blood, putrefaction of carbon monoxide blood takes place slowly. Even after 2 months in the case of an adult, who died from breathing coal gas and whose blood was saturated with carbon monoxide up to 68 per cent., the author got a distinct test for carbon monoxide. In this instance the blood had no odor whatever of putrefaction.

A. Chemical Detection

Most of the tests recommended for detection of carbon monoxide blood depend upon difference in behavior of oxyhaemoglobin and carboxyhaemoglobin toward certain reagents. Usually oxyhaemoglobin is decolorized, whereas the reagent has no effect upon the light red color of carboxyhaemoglobin. Certain of these reagents are sodium hydroxide solution, hydrogen sulphide, potassium ferrocyanide, tannic acid, alkaline pyrogallie acid solution, and salts of heavy metals.

1. **Boiling Test.**—Blood containing carbon monoxide gives a brick-red coagulum, if boiled or warmed upon the water-bath. Ordinary blood gives a grayish brown or brownish black precipitate.

2. **Sodium Hydroxide Test.**—Carbon monoxide blood shaken with 1-2 volumes of sodium hydroxide solution (sp. gr. 1.3 = 26.8 per cent.) gives a red coagulum of carbon monoxide haematin. Normal blood similarly treated gives gelatinous oxyhaematin having a green-black to black color and in a thin coating upon a porcelain plate dark greenish brown. According to Salkowski, the best way to make this test is to dilute the blood in a test-tube with 20 times its volume of water and then add an equal volume of sodium hydroxide solution (sp. gr. 1.34). Carbon monoxide blood at first gives a milkiness and then becomes light red, whereas normal blood is changed to a dirty brown mixture.

3. **Basic Lead Acetate Test.**—Mix 4-5 volumes of basic lead acetate solution in a test-tube with diluted or undiluted carbon monoxide blood and shake well for several minutes. Such blood remains

bright red but normal blood is first brownish and then chocolate to greenish brown

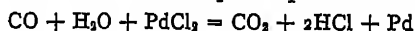
4. **Potassium Ferrocyanide Test.**—Mix undiluted blood (15 cc.) with an equal volume of 20 per cent. potassium ferrocyanide solution and 2 cc. of dilute acetic acid (1 vol. of glacial acetic acid + 2 vols. of water). Shake the mixture gently and a coagulum will gradually form. That from normal blood is dark brown but bright red from blood containing carbon monoxide. This difference disappears slowly but not entirely for weeks

5. **Tannic Acid Test.**—Mix a solution of 1 part of blood in 4 parts of water with 3 times its volume of 1 per cent. tannic acid solution and shake well. A difference in color between normal and carbon monoxide blood may be recognized after several hours, most distinctly after 24 hours. Normal blood is gray but carbon monoxide blood is crimson-red. This difference is apparent even after several months. Ten per cent. of carboxyhaemoglobin may be detected in blood by tests 4 and 5.

6. **Copper Sulphate Test.**—A drop of saturated copper sulphate solution added to 2 cc. of carbon monoxide blood mixed with the same volume of water gives a brick-red precipitate. The deposit from normal blood is greenish brown. In all these precipitation tests (4, 5 and 6) the less easily decomposed carbon monoxide blood remains bright red but the more easily decomposed normal blood in presence of the precipitants used and others is off color or dark.

7. **Ammonium Sulphide Test.**—Mix 0.2 cc. of ammonium sulphide solution and 0.2–0.3 cc. of 30 per cent. acetic acid with 10 cc. of 2 per cent. aqueous blood solution. Carbon monoxide blood gives a fine rose color but normal blood is greenish gray. Within 24 hours the former gives a red flocculent precipitate. In the procedure of Salkowski, 20–24 drops of blood are added to 50 cc. of water and then half to three-fourths its volume of saturated hydrogen sulphide water. Carbon monoxide blood does not change color but normal blood gives a dirty green mixture owing to formation of thiohaemoglobin.

8. **Palladous Chloride Test.**—Carbon monoxide precipitates black metallic palladium from a neutral aqueous palladous chloride solution:



Mix a few drops of potassium hydroxide solution with the blood and warm gently upon the water-bath. By means of a suction-pump, draw through the solution air that has been washed until pure.

Pass the gas evolved first through lead acetate solution to remove possible hydrogen sulphide, then through sulphuric acid to absorb ammonia and finally through a neutral light red palladous chloride solution (1:500).

Notes.—Franzen and v. Mayer¹ have examined the various tests for detection of carbon monoxide in blood and have come to the conclusion that the best are Hoppe-Seyler's sodium hydroxide test as modified by Salkowski, and the ferrocyanide and tannic acid tests carried out according to Kunkel and Welzel. All three tests make it possible to detect 1 per cent of carbon monoxide in blood with certainty. The color change in the tannic acid test appears only after several hours, whereas the sodium hydroxide and potassium ferrocyanide tests may be seen very clearly even after a short time. For this reason the two latter tests deserve first consideration in the practical detection of small quantities of carbon monoxide.

B. Spectroscopic Detection

Detection of carboxyhaemoglobin by the spectroscope is comparatively easy. The two absorption-bands of this compound are quite similar to those of oxyhaemoglobin but lie somewhat nearer together and more toward the violet. The main difference, however, between the absorption-bands of these compounds is that those of carboxyhaemoglobin are not extinguished by reducing agents. To prepare the blood solution for spectroscopic examination, dilute 1-1.5 parts of blood with 100 parts of water and make the observations through a layer 1 cm. thick. To reduce 1 per cent blood solution, mix well with 1-2 cc. of ammonium sulphide solution and add 4-6 drops more of the same reagent as a surface-layer to exclude air. In the case of normal blood, reduction begins in about 2-3 minutes. A solution of tartaric acid and ferrous sulphate in presence of excess of ammonium hydroxide solution will also reduce oxyhaemoglobin.

Under these conditions oxyhaemoglobin is changed to reduced haemoglobin. The two absorption-bands characteristic of the former disappear and a broad diffuse absorption-band occupies the previous bright space between the two bands. The spectrum of carboxyhaemoglobin remains unchanged only when 26.5 per cent at least of the haemoglobin is saturated with carbon monoxide. If allowed to stand in an open vessel, blood will lose carbon monoxide within 8 days. But carbon monoxide blood sealed in glass tubes is said to keep for years. Carbon monoxide has been detected spectroscopically in blood of a cadaver after 18 months.

¹ H. Franzen and O. v. Mayer. Detection of Carbon Monoxide with Blood. *Zeitschr. f. analyt. Chem.* 50 (1911), 669.

Tollens¹ recommends adding formaldehyde to the blood solution to be examined spectroscopically. This reagent has not the slightest effect upon the two oxyhaemoglobin bands. Warming the mixture very gently with ammonium sulphide solution develops a third and nearly as distinct black band almost midway between the original bands which gradually disappear. Finally only this band will remain. This is a far more satisfactory test than that given by the indefinite band of blood alone. If the solution is cooled and agitated with air, this third band will disappear and the two original oxyhaemoglobin bands return.

In presence of carboxyhaemoglobin, formaldehyde does not have this action. Then upon warming with ammonium sulphide the two bands of carboxyhaemoglobin displaced somewhat toward the oxyhaemoglobin bands remain even after

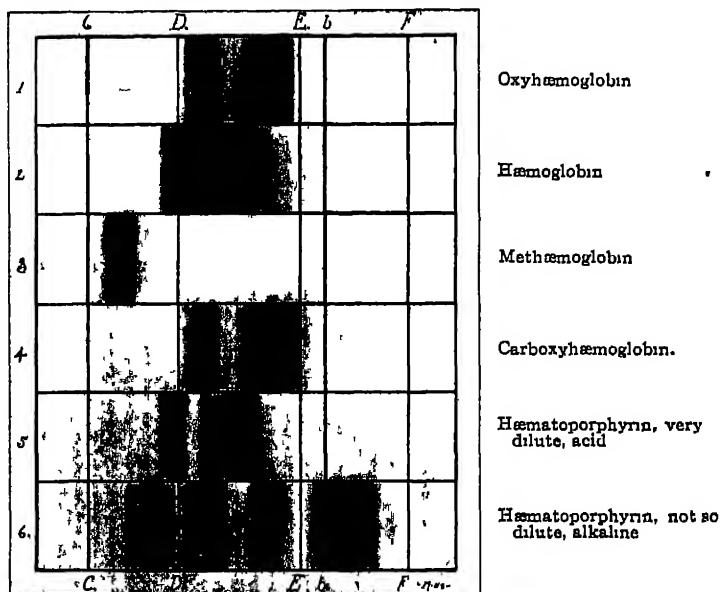


FIG. 27 —Absorption-spectra

addition of formaldehyde. Mix 15-30 drops of blood with 100 cc. of water, adding always a few drops of yellow ammonium sulphide. Then add 3-4 drops of approximately 40 per cent formaldehyde solution either before or after ammonium sulphide.

C. Approximately Quantitative Estimation of Carboxyhaemoglobin in Carbon Monoxide Blood

Frequently the forensic chemist is called upon to decide whether death due to poisoning from inhalation of illuminating gas, or coal

¹ R. Tollens: Spectroscopic Test for Blood in Presence of Formaldehyde. Ber. d. Deutsch. chem. Ges. 34 (1901), 1426

gas, should be ascribed exclusively to poisoning from carbon monoxide. The answer to this question depends upon the quantitative estimation of carboxyhaemoglobin, since according to statements made above blood must be saturated with carbon monoxide to a definite degree before death from asphyxiation occurs. In an investigation of this character, the author adopts Welzel's simple method which gives a result quickly and is of sufficient accuracy for practical purposes. Experiments with blood containing a known quantity of carbon monoxide have shown that blood containing not over 26.5 per cent. of carbon monoxide behaves spectroscopically toward reducing agents exactly like normal blood, that is, shows a broad diffuse band. Consequently, if enough normal blood is added to the blood in which carboxyhaemoglobin is to be determined just to produce the result mentioned, the quantity of carboxyhaemoglobin may be calculated from the quantity of normal blood required. Experiments with this method showed that rabbits die when their blood is three-quarters saturated with carbon monoxide.

II. Detection and Estimation of Carbon Monoxide in Air

Of the many methods recommended for detection of carbon monoxide in air, the two most likely to give a reliable result are. I. That depending upon formation of carboxyhaemoglobin and its spectroscopic recognition; II. That based upon the reduction of palladous chloride solution by carbon monoxide

Method I.—Shake one or several liters of air with about 5 cc. of an aqueous solution of normal blood, diluted so that it still appears to have a faint red color and yet permits the normal blood spectrum to be distinctly recognized. In presence of carbon monoxide, the blood solution changes color and at the same time behaves differently when examined spectroscopically after addition of ammonium sulphide. This test may be rendered more delicate by drawing 10–20 liters of suspected air by an aspirator through a few cc. of diluted blood solution and then examining it spectroscopically. These directions given by Hempel permit detection of even 0.05 per cent. of carbon monoxide in air. By passing the gas through two glass funnels held together by a rubber ring and placing a live mouse in the enclosure thus formed, Hempel has rendered this method exceedingly delicate. The gas is passed through for 3–4 hours at the rate of 10 liters per hour. The mouse is killed by dipping the funnels

under water. A few drops of blood are taken from the region of the heart, diluted with water and tested spectroscopically. In this manner Hempel has detected with certainty even 0.032 per cent of carbon monoxide. No toxic symptoms from these small quantities of carbon monoxide are visible. They do not appear until the quantity of carbon monoxide is 0.06 per cent and then after about half an hour. The mouse has dyspnoea and lies on its side exhausted. This test for carbon monoxide may be negative in presence of acid vapors and even of considerable quantities of carbon dioxide. For this reason the gas before entering the funnels should be passed through 2-3 wash-bottles filled with potassium hydroxide solution.

Method II.—Pass about 20 liters of air through 50 cc. of diluted blood. Immediately heat this blood solution in a flask at 90-95° and draw slowly through for 3-4 hours a current of air washed through dilute palladous chloride solution. Oxygen of the air by mass action gradually displaces carbon monoxide from its union with haemoglobin. The current of air containing carbon monoxide after leaving the blood solution is purified by being drawn first through dilute sulphuric acid, then through lead acetate solution, and finally through neutral, dilute palladous chloride solution having a light, yellow-red color. If air contains carbon monoxide, a black precipitate of metallic palladium appears. Gaglio states that even rather old blood will give useful results, if pieces of potassium hydroxide, or its solution, are added. The advantage of this method is that blood then does not coagulate. A more rapid method of detecting carbon monoxide in air is to use palladous chloride paper. This is moistened and suspended in a flask containing some water and filled with the given air. The flask for this purpose should have a volume of at least 10 liters. Even after a few minutes 0.05 per cent. of carbon monoxide gives a black, shiny film upon the paper; 0.01 per cent. after about 4 hours; and 0.001 per cent. after 20-24 hours. Sensitive as this test is, it is not characteristic, since hydrogen sulphide, ammonia, other components of illuminating gas, and also sunlight cause darkening of palladous chloride paper. Consequently this test is significant only when negative.

Gadamer's¹ Quantitative Estimation of Carbon Monoxide

To obviate the disadvantages mentioned, Gadamer first absorbs carbon monoxide in hydrochloric acid solution of cuprous chloride

¹ J. Gadamer: *Lehrbuch der chemischen Toxikologie* 1909, page 46

and then allows this product to react with palladous chloride solution. The precipitate of metallic palladium is finally weighed.

Procedure.—Wash a measured volume of air with lead acetate solution and then pass through hydrochloric acid solution of cuprous chloride containing finely divided metallic copper. Filter into palladous chloride solution, disregarding any precipitate that appears, and add ammonia in excess. Metallic palladium separates almost at once and copper salts pass into solution. Excess of palladous chloride is of course required. Collect the palladium precipitate in a weighed asbestos filter-tube, or Gooch crucible, wash with ammonia, and ignite after having dried first in a stream of hydrogen and then in air. Finally weigh crucible and palladium.

Calculation.—According to the equation $\text{PdCl}_2 + \text{H}_2\text{O} + \text{CO} = \text{Pd} + 2\text{HCl} + \text{CO}_2$, 1 mg of palladium corresponds to 0.263 mg of carbon monoxide calculated from Pd (106.5) CO (28) = $1 \times (x = 0.263)$

Under normal conditions a molecular volume of carbon monoxide occupies a volume of 22400 cc. According to the equation $28 \times 22400 = 0.000263 \times (x = 0.2104)$, 1 mg of palladium corresponds to 0.2104 cc of carbon monoxide at 0° and 760 mm.

According to Gadamer, this method of estimation is not entirely free from objection, since occasionally, for reasons as yet unexplained, even cuprous chloride solution alone causes precipitation of palladium. To avoid error, Gadamer recommends always making a blank test with air free from carbon monoxide under precisely the same conditions that obtain in the actual test.

B. ORGANIC SUBSTANCES

Organic Arsenical Compounds

In general the toxic action of arsenic in inorganic combination, known also as "mineral arsenic," upon man and higher animals is considerably greater than that of the same quantity of arsenic in organic combination. The minimal lethal dose of As_2O_3 , alkali arsenites and sodium arsenate (Na_2HAsO_4) contains 0.0037 gram of arsenic per kilogram of rabbit, whereas for atoxyl the quantity is 0.072 gram. The dose, causing no actual acceleration of death upon further increase, contains 0.018 gram of arsenic for the inorganic arsenic compounds mentioned per kilogram of rabbit, whereas for atoxyl the quantity is 0.2 gram. Certain organic arsenical compounds, because of lower toxicity for man, have for a number of years found use in medicine, especially in those cases where prolonged action from arsenic administered by the mouth, subcutaneously, or intravenously is desired. The substances to be considered

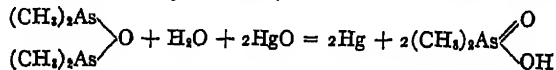
are derivatives of quinquivalent arsenic, that is, of arsenic acid (H_3AsO_4) through substitution of organic radicals for one or two hydroxyl groups. The most important compounds of this series because of use in medicine are:

- 1 Arrhenal, or Sodium Methyl Arsinat, $\text{CH}_3 \text{AsO}(\text{ONa})_2 \cdot 6\text{H}_2\text{O}$
- 2 Sodium Cacodylate, or Sodium Dimethyl Arsinat, $(\text{CH}_3)_2\text{AsO}(\text{ONa}) \cdot 2\text{H}_2\text{O}$
- 3 Atoxyl (Arsanilate), or Sodium Para-arsanilate, $\text{H}_2\text{N C}_6\text{H}_4 \text{AsO}(\text{ONa})(\text{OH}) \cdot 4\text{H}_2\text{O}$
- 4 Arsacetine, or Sodium Acetyl-para-arsanilate, $\text{CH}_3 \text{CO NH C}_6\text{H}_4 \text{AsO}(\text{ONa})(\text{OH}) \cdot 4\text{H}_2\text{O}$
- 5 Salvarsan, Ehrlich-Hata 606, or 3,3'-Diamino-4,4'-dihydroxy-arsenobenzene Dihydrochloride, $\text{HCl NH}_2 \text{C}_6\text{H}_3(\text{OH}) \text{As} = \text{As C}_6\text{H}_3(\text{OH}) \text{NH}_2 \text{HCl}$

Organic compounds of arsenic most commonly used in medicine subcutaneously, or intravenously, such as atoxyl and cacodylic acid, are for the most part eliminated unchanged rather quickly by way of the urine. For complete elimination, however, usually several months are required. In the case of several persons who had taken the salvarsan treatment and died 2-5 months after the last injection, the author found in the liver determinable quantities of arsenic. In view of the extensive use of salvarsan by luetics, the forensic chemist should proceed with the greatest caution in expressing an opinion concerning a positive result for arsenic. Having found arsenic in any part whatsoever of the cadaver, he should not decide that it is due to arsenical poisoning until he has acquainted himself with every detail of the case. It especially becomes his duty to ascertain whether arsenic found may not have been acquired from therapeutic use of salvarsan, atoxyl, or some other organic compound of arsenic. Such medical use, however, may not have been of recent date.

Cacodylic Acid

Bunsen¹ prepared cacodylic acid, $(\text{CH}_3)_2\text{AsO OH}$, by oxidizing cacodyl, or better cacodylic oxide, under water with mercuric oxide:



The solution filtered from mercury is evaporated to dryness with moderate heat and the residue recrystallized from hot alcohol.

Cacodylic acid forms colorless, inodorous, rhombic crystals, melting at 200° and readily soluble in water. The resistance it

¹ R. Bunsen: Researches upon Cacodyl Compounds. *Annal d Chemie und Pharm* 46 (1843), 2

offers to oxidation is extraordinary. Fuming nitric acid, aqua regia, chromic acid and potassium permanganate are without action. Potassium chlorate together with hydrochloric acid has no effect upon it. Phosphorous acid reduces it to cacodylic oxide. Cacodylic acid is monobasic, its salts for the most part being readily soluble in water. Sodium cacodylate, $(\text{CH}_3)_2\text{AsO ONa}$, is a white crystalline powder freely soluble in water. Mercuric cacodylate, $[(\text{CH}_3)_2\text{AsO.O}]_2\text{Hg}$, obtained by dissolving freshly precipitated yellow mercuric oxide in aqueous cacodylic acid and allowing the solution to evaporate spontaneously, forms fine, white, crystalline needles that easily decompose. Cacodylic acid and its salts are characterized by low toxicity. They are slowly eliminated unchanged in greatest part by way of the urine. Cacodylic acid with nascent hydrogen, especially with zinc and sulphuric acid, gives white fumes having the odor of cacodylic oxide and coloring silver nitrate solution red-brown. Arsine is not set free. This is not evolved unless cacodylic acid has previously been boiled for several hours with concentrated sulphuric acid. Cacodylic acid heated with magnesium powder gives off a strong odor of cacodyl.

Arsenic in inorganic combination is readily distinguished from cacodyl arsenic by the property possessed by cacodylic acid of not forming yellow arsenic sulphide with hydrogen sulphide even after treatment with potassium chlorate and hydrochloric acid, or other oxidizing agents. On the other hand, when examined in the Marsh apparatus, cacodylic acid gives a yellow-red ring characteristic of this arsenic compound. In the reduction tube, it also evolves dimethylarsine, $(\text{CH}_3)_2\text{AsH}$, still recognizable by its cacodyl odor even when only 0.01 mg. is present (Ganassini¹). Cacodylic acid, although extraordinarily stable toward oxidizing agents, when introduced in small quantities into fused potassium nitrate is converted for the most part into potassium arsenate.

Baumert's Modification of Vitali's² Procedure for Detection of Cacodylic Acid in Urine and Parts of the Cadaver

Acidify the material (urine and organs) with tartaric acid, evaporate to dryness upon the water-bath, and extract the residue with

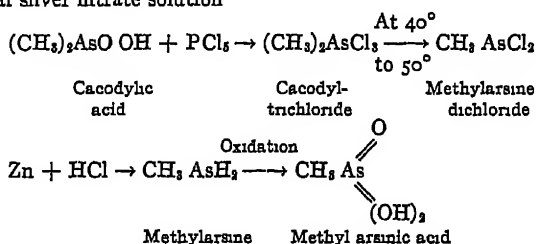
¹ D. Ganassini. Cacodylic Acid and Its Toxicological Detection. *Boll. Chimic. Farmacol.* 42 (1903), 5

² D. Vitali. Elimination of Cacodylic Acid and Its Detection in Poisoning Cases. *Boll. Chim. Farmacol.* 40 (1901), 657

90 per cent. alcohol. Filter this extract and distil off the alcohol. Add chloroform to the residue and sufficient alcohol to bring all the chloroform into solution. Addition of water to this alcohol-chloroform mixture precipitates cacodylic acid held in solution by chloroform. Separate this chloroform solution, distil off the chloroform, and test the residue for cacodylic acid. It appears as prismatic crystals readily soluble in water, methyl or ethyl alcohol, and chloroform. Aqueous solutions give the penetrating cacodyl odor with zinc and dilute sulphuric acid, phosphorous acid, hypophosphorous acid, and also with Bougault's reagent. The same odor is developed by fusion with potassium persulphate, or by heating with magnesium powder. Finally the solution of the residue may be examined in the Marsh apparatus.

Methyl Arsinic Acid and Arrhenal

Cacodylic acid is the best substance to use for the preparation of methyl arsinic acid. Treated with phosphorus pentachloride, it is converted into cacodyl-trichloride through replacement of O and OH by chlorine. Warmed to 40–50°, the latter loses methylchloride and is converted into methylarsine dichloride. This compound reduced by means of amalgamated zinc dust and alcoholic hydrochloric acid gives off methylarsine, an exceedingly poisonous gas. Methyl arsinic acid is formed from this gas by oxidation and passing it into neutral silver nitrate solution.



Methyl arsinic acid crystallizes from alcohol in colorless leaflets melting at 161°.

Arrhenal.—This compound is the disodium salt of methyl arsinic acid, $\text{CH}_3\text{AsO}(\text{ONa})_2 \cdot 6\text{H}_2\text{O}$, obtained by dissolving As_2O_3 and sodium hydroxide in a mixture of water and alcohol, adding methyl iodide, and allowing the clear mixture to stand in a closed vessel for several days.



Arrhenal forms colorless crystals, dissolving readily in water but with difficulty in alcohol. These crystals effloresce in air and so vary in the quantity of water they contain. An aqueous solution is alkaline and is precipitated by silver nitrate, copper sulphate, lead acetate and mercuric chloride. These precipita-

tion reactions serve to distinguish arrhenal from sodium cacodylate. Among other differences the following may be mentioned. In the Marsh apparatus arrhenal, in absence of platonic chloride, gives rise to black rings not preceded by appearance in the evolution flask of white vapor having a garlic odor, as is the case with cacodylic acid. Warmed with Bettendorff's reagent, cacodylic acid gives a white vapor and garlic odor but no coloration. Arrhenal gives no reaction, or only a yellow coloration. But on longer heating a white sublimate, later turning yellow, and finally becoming brown, forms upon the upper part of the tube.

Ten times the quantity of hypophosphorous acid (sp. gr. 1.15) with cacodylic acid at once produces an intense cacodyl odor, whereas arrhenal remains odorless.

Quantitative Estimation of Arsenic in Cacodylic Acid and Arrhenal

The difficulty attending the breaking up of the combination in case of these two organic arsenic compounds and reconvertng arsenic into an inorganic compound is very great. The method recommended by d'Emilio¹ certainly should bring about this change. Boil for about 1 hour over a free flame in a Kjeldahl flask 0.2-0.3 gram of substance with 10 cc. of arsenic-free, concentrated sulphuric acid and 10 grams of potassium sulphate, adding occasionally a little powdered potassium permanganate. Cool, dilute with water, neutralize with ammonia to faint acid reaction, and precipitate arsenic with hydrogen sulphide. Since the precipitate always consists of a mixture of As_2S_3 and As_2S_5 and free sulphur, it is advisable to dissolve the filtered and washed precipitate in ammonia upon the paper. Evaporate the solution in a porcelain dish and repeatedly evaporate the residue with strong bromine water to effect complete conversion of arsenious to arsenic acid. Finally precipitate arsenic acid thus obtained with magnesia mixture and weigh as magnesium pyroarsenate, $Mg_2As_2O_7$.

To estimate cacodylic acid in urine quantitatively, Heffter² evaporates the latter rendered alkaline with sodium hydroxide solution and gradually introduces the residue, mixed with 1 part of potassium hydroxide and 5 parts of potassium nitrate, into a glowing crucible. Dissolve the cold melt in water and excess of hydrochloric acid and boil for some time, to remove nitrous acid completely and most of the nitric acid. Then precipitate arsenic with hydrogen sulphide, convert the precipitate as directed above into arsenic acid,

¹ L. d'Emilio. Organic Arsenic in Therapy. Cacodylic Acid, Methyl Arsenic Acid and Its Salts. *Boll. Chim. Farm.* 41 (1902), 633.

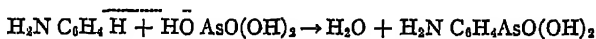
² A. Heffter. Behavior of Cacodylic Acid in the Organism and Detection in Urine. *Schweiz. Wochenschr. Pharm.* 39 (1901), 193.

and finally weigh magnesium pyroarsenate, $\text{Mg}_2\text{As}_2\text{O}_7$. In the opinion of many authors, large quantities of cacodylic acid escape oxidation by the fusion mixture of potassium hydroxide and potassium nitrate and for this reason Heffter's method gives too low results.

Notes—After the last dose of cacodylic acid administered by the mouth, elimination by way of the urine continues for a long time. In fact it has been observed to be taking place after 70 days. The cacodyl odor of the urine is about the best means of recognizing presence of cacodylic acid (Barthe and Péry¹).

Atoxyl

Atoxyl is the sodium salt of para-arsanilic acid, $\text{H}_2\text{N} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}(\text{OH})(\text{ONa}) \cdot 4\text{H}_2\text{O}$, formed by heating a mixture of aniline and arsenic acid, consequently aniline arsenate, up to $180\text{--}200^\circ$ with elimination of water.



Dissolve the product of this reaction in sodium carbonate solution, filter and saturate with hydrochloric acid. Free para-arsanilic acid is precipitated and recrystallized from hot water. The shining white needles thus obtained are soluble with difficulty in cold water and in alcohol, easily soluble in boiling water and in methyl alcohol, and almost insoluble in ether, chloroform and acetone. Neutralization of free acid with one equivalent of sodium carbonate or bicarbonate gives atoxyl. The free acid, however, also has weak basic properties, for with hydrochloric acid it forms the salt $\text{HCl} \cdot \text{H}_2\text{N} \cdot \text{C}_6\text{H}_4 \cdot \text{AsO}(\text{OH})_2$.

Atoxyl is a white, crystalline, inodorous powder, soluble in about 6 parts of water at 17° and very freely soluble in hot water with neutral reaction. Almost insoluble in alcohol, it is easily soluble when anhydrous in methyl alcohol. Presence of a free amino-group in atoxyl explains the solubility of free para-arsanilic acid in dilute mineral acids, the easy formation of an acetyl derivative, and conversion by means of hydriodic acid into para-iod-aniline.

Therapeutic Use.—As compared with inorganic arsenic compounds, atoxyl is characterized by low toxicity. It is a specific for sleeping sickness and to some extent also for lues. It is further recommended in treatment of skin diseases, anaemia and tuberculosis.

¹L. Barthe and R. Péry: Elimination and Toxicological Detection of Cacodylic Acid. *J. Pharm. Chim.* (6) 13 (1901), 209.

Detection of Atoxyl¹

A. Arsenic.—Carefully heated in a fusion tube, atoxyl gives a garlic-like odor and an arsenic mirror. Hydrogen sulphide passed for a long time into a hot solution of atoxyl precipitates first sulphur and then yellow arsenic sulphide. In the Marsh and Gutzeit tests it behaves like inorganic arsenic compounds. The Reinsch test also gives a distinct result though rather slowly. Consequently atoxyl cannot be present when these three tests are negative! On warming, Bettendorff's and Bougault's reagent gradually give lemon-yellow but not brownish precipitates. Distilled according to Schneider-Fyfe-Beckurts in a stream of hydrochloric acid, atoxyl does not give arsenic trichloride. It will yield the latter, however, when distillation takes place in presence of ferrous chloride.

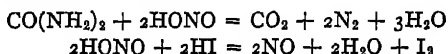
B. The Primary Aromatic Amino-Group.—As a primary aromatic amine, atoxyl can be diazotized and made to form azo-dyes by coupling its diazonium salt with phenols, such as resorcinol and β -naphthol, and also with α - and β -naphthylamine. For example, diazotized p-arsanilic acid gives with β -naphthylamine a red, crystalline precipitate of the azo-dye $\text{HCl NH}_2 \cdot \text{C}_{10}\text{H}_7\text{N} = \text{N C}_6\text{H}_4 \text{AsO}(\text{OH})_2$,² readily soluble with red color in cold sodium carbonate solution and reprecipitated from this solution by acids.

Procedure.—Add a few drops of sodium nitrite solution (1:10) to an aqueous atoxyl solution, cool, acidify with dilute hydrochloric or sulphuric acid, and add to this diazonium solution a few drops of a hydrochloric acid solution of α - or β -naphthylamine. The α -derivative gives a purple-red color and the β -derivative more of a brick-red color that soon forms a colored precipitate leaving the solution nearly colorless. Atoxyl may also be precipitated quantitatively by means of the latter reaction. Neither reaction, however, in pure aqueous solutions is characteristic, since the naphthylamines themselves even in absence of atoxyl give purple-red colorations due to diazotization of a part of the base and self-coupling. Consequently after diazotization of aqueous atoxyl solutions, or aqueous extracts of organs, sufficient urea must be added to decompose all

¹ J. Gadamer: Atoxyl in the Forensic Detection of Arsenic. *Apotheker-Zeitung* 22 (1907), 566.

² P. Ehrlich and A. Berthelm: Para-arsanilic Acid. *Ber. d. Deutsch. chem. Ges.* 40 (1907), 3292.

free uncombined nitrous acid, as shown by failure to produce a blue color on starch iodide paper.



Then add the naphthylamine solution. This reaction may be applied to urine direct, for it contains enough urea to take care of any excess of nitrous acid in the diazonium solution. An alkaline resorcinol or β -naphthol solution, added to diazotized atoxyl solution so that the reaction is alkaline in the end, produces a purple-red color due to formation of an azo-dye. Although these tests are somewhat less sensitive, they are more reliable and more characteristic than those given by the naphthylamines

The reactions under (B) merely show presence of a primary aromatic amino-compound. They are conclusive for presence of atoxyl or salvarsan only when the azo-dye is precipitated, washed and shown to contain arsenic. To make this test, filter the brick-red precipitate obtained by means of the solution of β -naphthylamine hydrochloride, wash, dry, and by fusion with sodium nitrate, or magnesium oxide (see Chapter V, page 493), convert arsenic into arsenic acid, testing the product in the Marsh apparatus. If sodium nitrate was used for the fusion, first completely expel nitric and nitrous acids from the solution of the melt by heating with arsenic-free sulphuric acid until copious white fumes are given off. Gadammer detects atoxyl in urine and parts of the cadaver in the following manner. Having decolorized strongly colored urine with a little freshly ignited animal charcoal, acidify with sulphuric acid, cool, and test for formation of azo-dye by reactions given above under (B). Atoxyl is not present, unless dyestuff is formed. If the test is positive, frequently a further test must be made to determine whether mineral arsenic (arsenious or arsenic acid) is not present too. To do this, completely precipitate atoxyl with β -naphthylamine as under (B), and test the filtrate for arsenic in the usual manner after having first destroyed organic matter with hydrochloric acid and potassium chlorate.

Comminute organs, or other parts of the cadaver, add to the material many times its volume of alcohol, render just acid with sulphuric acid, and warm for several hours. Filter, evaporate the solution, gradually but completely precipitate the syrupy residue with absolute alcohol, and expel alcohol from the filtered solution by distillation or evaporation. Dissolve the residue in water and examine the filtered solution as follows

- 1 First test for arsenic by means of the Reinsch, Marsh or Gutzeit test. If the test is negative, atoxyl is absent.

- 2 If test (1) is positive, add 3 vols. of Bettendorff's reagent to 1 vol. of the solution and warm gently. If this reagent gives no reaction, or only separation of a lemon-yellow precipitate, atoxyl may be present but no mineral arsenic. To test with greater certainty for atoxyl, try the diazo-reactions given under (B). In tests with naphthylamines excess of nitrous acid should be removed by means of urea before naphthylamine hydrochloride solution is added. Arsacetine, or

acetyl-atoxyl, does not give the diazo-reaction until after treatment with strong hydrochloric acid or sodium hydroxide solution.

If Bettendorff's reagent produces a brown precipitate with the original solution, mineral arsenic is present

Elimination of Atoxyl in Urine.—Croner and Seligmann¹ found that atoxyl after subcutaneous injection was eliminated very quickly mainly through the urine, that is, within 24 hours, and almost wholly unchanged. For days traces of atoxyl may be detected in urine. Elimination of atoxyl is prolonged after injections repeated at short intervals. The azotizing method of Ehrlich and Butheim may be used to detect and estimate atoxyl in urine. With β -naphthylamine the diazonium compound of atoxyl gives a carmine-red precipitate having the composition, $\text{HCl NH}_2\text{C}_{10}\text{H}_8 \text{N} = \text{N C}_6\text{H}_4 \text{AsO}(\text{OH})_2$. Addition of sodium acetate solution forms, instead of the hydrochloride, the free amino-compound having a more purple-red color.² The limit of delicacy of atoxyl detection by precipitation as azo-dye is 1 mg. in 100 cc. of water, or a dilution of 1:100,000. In case of urine this proportion does not always hold, since presence of other substances interferes. Elimination of atoxyl after subcutaneous injection in man is nearly at an end after 9 hours. Within this period in individual cases atoxyl as such leaves the organism almost quantitatively. Usually elimination lies between 50-90 per cent. Part of the atoxyl taken undergoes a change within the human body. Elimination of arsenic present in urine not as atoxyl may be of several days duration (Igersheimer and Rothmann³). These authors found that atoxyl dissolved in the serum circulates through the body but that apparently the corpuscles have only a slight affinity for the poison. Appreciable quantities of a reduction product of atoxyl are not present in urine but most of this compound is eliminated as such.

Acetyl-Para-Arsanilic Acid and Arsacetine⁴

A mixture of sodium p-arsanilate (=atoxyl) and acetic anhydride becomes warm when stirred and actively boils. Momentarily everything passes into solution and then solidifies to a mass of crystals. Stir with dilute hydrochloric acid when cold, suck off the mother-liquor after several hours, and wash with water, alcohol and ether. Acetyl-arsanilic acid, or acetyl-p-amino-phenyl-arsinic acid, $\text{CH}_3\text{-CO.NH C}_6\text{H}_4 \text{AsO}(\text{OH})_2$, thus obtained forms shining, white leaflets, has scarcely any basic properties, and so is precipitated unchanged by water from solution in concentrated hydrochloric acid. It may also be prepared by boiling atoxyl for several hours with glacial acetic acid. By introducing acetyl-arsanilic acid into warm concentrated sodium hydroxide solution to the point of neutralization, its sodium salt, arsacetine, $\text{CH}_3\text{CO.NH C}_6\text{H}_4 \text{AsO}(\text{ONa})(\text{OH})_5\text{H}_2\text{O}$, crystallizes as the solution cools in fine, white needles easily soluble in water and in methyl alcohol. Precipitation of the aqueous solution with alcohol gives an arsacetine crystalliz-

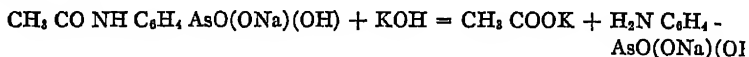
¹ Croner and Seligmann. *Deutsche med. Wochenschrift* 33, 995.

² G. Lockemann and M. Paneke. *Detection and Process of Elimination of Atoxyl in Urine*. *Deutsche med. Wochenschrift* 34 (1908), No. 34.

³ J. Igersheimer and A. Rothmann. *Behavior of Atoxyl in the Organism. Quantitative Estimation of Atoxyl*. *Zeitschr. f. physiol. Chemie* 59 (1909), 256.

⁴ P. Ehrlich and A. Berthelm. *Para-arsanilic Acid*. *Ber. d. Deutsch. chem. Ges.* 40 (1907), 3292.

ing with $4\text{H}_2\text{O}$ Boiling caustic alkalis hydrolyze it, forming arsanic acid and acetic acid



Introduction of the acetyl-group greatly diminishes the toxicity of atoxyl, farsacetine is considerably less toxic than atoxyl At the same time Ehrlich found it to be equal to the latter in curative powers for many animal species Solution of farsacetine sterilized in autoclave at 130° undergo no decomposition

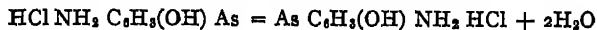
Estimation of Arsenic in Atoxyl and Farsacetine

(Rupp and Lehmann¹)

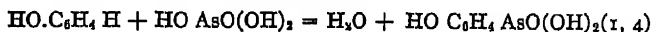
Warm 0.2 gram of material at 70° in a 200 cc. flask with 10 cc. of concentrated sulphuric acid. Then while shaking the contents add in small portions at a time 1 gram of crystallized potassium permanganate. The mixture is decolorized by dropping in 5-10 cc of official 3 per cent. hydrogen peroxide Dilute with 20 cc of water, boil for 15 minutes, and add 50 cc. more of water. Cool and add 2 grams of potassium iodide Allow to stand for 1 hour and titrate free iodine without use of indicator

Salvarsan

Salvarsan, or Ehrlich-Hata 606, is 3,3'-diamino-4,4'-dihydroxyarseno-benzene dihydrochloride, having the composition:



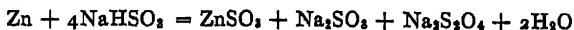
Preparation.—Phenol heated with sulphuric acid is converted into phenol-p-sulphonic acid and similarly treated with arsenic acid is changed to phenol-p-arsinic acid:



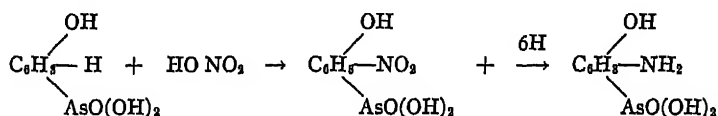
By the combined action of nitric and sulphuric acid at 0° this arsenic acid is nitrated to nitro-phenol-arsinic acid which like all nitro-derivatives is reduced by nascent hydrogen, best by sodium hydrosulphite² in alkaline solution, or by meta

¹ E Rupp and F Lehmann Simplified Estimation of Arsenic in Atoxyl and Farsacetine Apotheker-Zeitung 26 (1911), 203

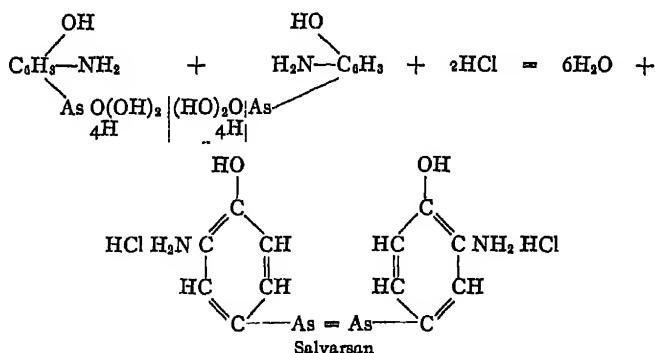
² Sodium hydrosulphite is the sodium salt of hydrosulphurous or dithionous acid. The sodium salt is formed by treating a concentrated solution of sodium acid sulphite with zinc filings In the formation of the free acid, or of the sodium salt, no hydrogen is evolved It is a very unstable compound, a strong reducing agent, rapidly absorbs oxygen from the air, becoming sulphurous acid or a sulphite According to Bernthsen (Ber d Deutsch. chem Ges 1 (1881), 438), the sodium salt does not contain hydrogen. He gives the formula as $\text{Na}_2\text{S}_2\text{O}_4$:



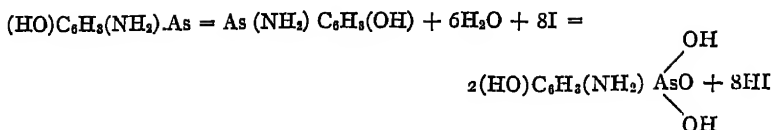
lic sodium in methyl alcohol solution, to amino-phenol-arsinic acid difficultly soluble in water



Further reduction of amino-phenol-p-arsinic acid with sodium hydrosulphite, or more energetic reduction of the original nitrated acid with a larger quantity of hydrosulphite, gives diamino-dioxy-arseno-benzene as a light yellow, crystalline, easily changeable powder. As a di-primary base this compound is soluble in dilute acids and as a diacid phenol is soluble in aqueous solutions of the caustic alkalis. Salvarsan is the dihydrochloride of diamino-dioxy-arseno-benzene



Salvarsan is a light yellow, crystalline powder slowly dissolving in water with acid reaction. It is easily soluble in methyl alcohol and in glycerol, somewhat more difficultly soluble in alcohol (1:12), and insoluble in ether. It contains 31.6 per cent of arsenic. Hydrogen sulphide fails to precipitate arsenic sulphide from salvarsan solutions acidified with hydrochloric acid. Bettendorff's reagent gives merely a yellow, amorphous precipitate but not brown-black arsenic. Warmed with hydrochloric acid and potassium chlorate, until the solution is water-clear, salvarsan then gives the arsenic reactions with these two reagents. By the Marsh, Gutzzeit, Schneider and Fyfe, Reinsch, and also biological test, it is possible to show presence of arsenic in salvarsan. In salvarsan solutions ferric chloride produces a coloration of green to red even in dilution of 1:15,000. Gold chloride solution gives at once an intense red coloration. Iodine solution oxidizes salvarsan to amino-phenol-arsinic acid.



Since 8 atoms of iodine to 1 molecule of salvarsan enter into this reaction, it may be employed to determine it volumetrically. As an aromatic, diprimary amine, salvarsan gives the diazo reaction. Unlike atoxyl, however, it fails to give this test with β -naphthylamine though it does with α -naphthylamine. To make the diazo test, acidify the salvarsan solution, with a few drops of hydrochloric acid, cool to 0° , add a slight excess of 0.5 per cent. sodium nitrite solution, removing nitrous acid by adding urea in small portions at a time until a drop of the solution no longer gives a blue color with starch-iodide paper. Finally add a saturated aqueous solution of α -naphthylamine acidified with hydrochloric acid. The beautiful ruby to violet-red coloration that appears increases in intensity on longer standing. Formation of this dyestuff is considerably hastened by heat. Salvarsan in dilution of 1:15,000 gives the red coloration in the cold after several hours. This diazo-reaction needless to say is conclusive for salvarsan only when presence of arsenic in the original solution, or better in the dyestuff formed, is established at the same time. To do this, saturate the red solution with salt as soon as gentle warming causes no further increase in intensity of color. The dyestuff is precipitated and after several hours may be filtered off, washed with saturated salt solution, dissolved in hot dilute hydrochloric acid, and tested by the Reinsch or Gutzeit test for arsenic.

Fate of Salvarsan in the Human Organism.—Shortly after administration, salvarsan is eliminated through the kidneys in the urine. But elimination of arsenic (I) does not cease for weeks. In the latter stage considerable quantities of arsenic always remain longer in the liver, and smaller quantities in the kidneys and spleen. Following intramuscular injection, arsenic is also stored in the injected muscle. In salvarsan poisonings terminating fatally the author has failed to find any arsenic in the brain, or at most traces. Examination of parts of the body for arsenic followed the usual forensic methods, organic matter being most effectively destroyed by means of hydrochloric acid and potassium chlorate. Diazotized salvarsan may of course be coupled to form azo-dyestuffs not only with α -naphthylamine but with phenols, such as resorcinol, phloroglucinol, α - and β -naphthol, as well as orcinol¹. Resorcinol and orcinol give the most beautiful dyestuffs. An aqueous solution of salvarsan treated with Ehrlich's

¹ Orcinol is 1-Methyl-3, 5-dioxy-benzene.

diazo-reagent, that is, a mixture of a hydrochloric acid solution of sulphanilic acid and sodium nitrite solution, and a few drops of ammonia gives a brown-red coloration. Caution is necessary, since the urine of patients treated with salvarsan gives with the Ehrlich diazo-reaction a color that is only slightly different in shade. The other substances in urine do not interfere with the resorcinol test of diazotized salvarsan. After diazotization atoxyl gives an orange color with resorcinol.

In view of the extensive therapeutic use of salvarsan, the forensic chemist should bear in mind that a considerable quantity of arsenic may be found in a cadaver. He should also take into account the fact that the human body may retain arsenic for quite a long time after its use medicinally in the form of an organic compound. Müller¹ cites a poisoning case where a person was found dead two days after intravenous injection of 0.5 gram of salvarsan. The stomach-contents contained not a trace of arsenic though it was found in other organs. Since the dose of salvarsan may run as high as 1 gram, the body will receive 0.34 gram of arsenic. But elimination of arsenic may continue for months. Not infrequently in legal cases the question may come up whether arsenic found in a cadaver came from salvarsan, or from an inorganic compound containing arsenic. In such cases it should be ascertained whether arsenic can be found in the stomach-contents and further whether salvarsan as such can be detected in the urine. The author has repeatedly examined the urine following intravenous injections of the usual doses of salvarsan or neo-salvarsan. He has found that the resorcinol test, when it appears at all, is entirely negative after a very short time, on the average after 6 hours! Colorimetric determinations have then shown that elimination of salvarsan as such, or in the form of a component (?) permitting formation of azo-dyestuff, is decreasingly small and always amounts to only a few mg. taken as salvarsan! To test for salvarsan, acidify 5-10 cc. of urine with hydrochloric acid, add a few drops of 0.5 per cent. sodium nitrite solution, and gradually add this mixture to a solution of 0.3 gram of resorcinol in about 5 cc. of water, to which 2-3 cc. of 20 per cent. sodium carbonate solution have been added. If the urine contains salvarsan, the resorcinol solution will turn red. Otherwise only a yellow color appears. In cases of sudden death the forensic chemist by means of this reaction can detect salvarsan still present in the urine, unless of course the salvarsan was injected too long ago. Directions given by Abelin² for detecting salvarsan in urine are almost identical with those above. He acidifies a measured quantity of urine (about 8 cc.) in a test-tube with 6-8 drops of hydrochloric acid, cools well, adds 3-4 drops of 0.5 per cent. sodium nitrite solution, and pours a few drops of this mixture into about 5-6 cc. of a colorless, alkaline 10 per cent. resorcinol solution. The latter immediately turns red in presence of salvarsan. An especially good method of observing this color is to pour the urine-mixture carefully without shaking upon the resorcinol solution. The reaction-mixture is then allowed to stand and after some time a dark zone appears in the upper liquid. By this method urines that contain no salvarsan, and also free nitrous acid, give only a

¹ G. Müller. Vierteljahrsschr. f. gerichtl. Med. u. öffentl. Sanitätsw. (3) 49 (1915), 48.

² L. Abelin. A New Method of Detecting Salvarsan. Münch. med. Wochenschr. 1911, page 1002.

yellow color In case of atoxyl-urine treated in the same manner only an orange color appears

In the examination of parts of the cadaver of a man who had been treated with salvarsan, Richter¹ was able to detect arsenic only at the site of injection He extracted the given material with alcohol containing hydrochloric acid In the liver and spleen he was unable to find arsenic In six cases of salvarsan poisoning terminating fatally the author obtained different results He always found arsenic in the liver in appreciable quantities and less in the kidneys and urine, whereas the brain was free The liver of a man, who died 3 months after the last salvarsan injection, still contained 23.6 mg of arsenic

Notwithstanding the fact that elimination of arsenic in the urine has apparently ceased, an appreciable quantity deposited in the organs is still retained in the human body for months According to Bornstein,² after intravenous injection most of the arsenic in organic combination does not circulate freely in the blood but is deposited in the natural storehouses of the body, that is, in the liver, spleen and kidneys Likewise salvarsan absorbed from the sites of subcutaneous and intramuscular injections does not remain in the blood circulation but is stored in the organs mentioned The fact is also notable that a considerable quantity of arsenic following intramuscular injection remains for some time in the injected muscle In the case of a woman who died 36 days after an injection, Fischer and Hoppe³ found 0.01 gram of arsenic in the muscle In the case of a man who had been treated with 0.7 gram of salvarsan and had died 3 weeks after injection, Gaebel⁴ examined a piece of flesh from the back surrounding the site of injection, consisting of skin, subcutaneous cell-tissue and muscle The piece of flesh weighed 143 grams and contained in all 0.074 gram of arsenic, corresponding to 0.22 gram of salvarsan Gaebel states that unquestionably the quantity of arsenic retained in the characteristically colored area of the site of injection was somewhat greater, for the examination did not include the entire region.

Detection in Organic Material.—Stir the material with several times its volume of 96 per cent alcohol acidified with a few drops of hydrochloric acid. Then digest the mixture for several hours at gentle heat and finally filter. Gradually add absolute alcohol to the syrupy residue left upon evaporation of the filtrate with moderate heat until nothing more is precipitated. Again filter, evaporate the filtrate and dissolve the residue in a little water. Filter the aqueous solution, testing for arsenic by the Reinsch and Gutzeit tests and for salvarsan by the diazo-reaction mentioned above. Two salvarsan urines gave a very distinct reaction by the α -naphthylamine test with the unchanged urine direct.

Approximately Quantitative Estimation of Arsenic in Salvarsan

By the Schneider-Fyfe-Beckurts method, that is, by destroying organic matter with hydrochloric acid and potassium chlorate and

¹ R. Richter. Detection of Salvarsan in Parts of Cadavers. *Pharm. Ztg.* 56 (1911), 314.

² Bornstein. *Deutsche med. Wochenschr.* 1911, page 112.

³ Fischer and Hoppe. *Münch. med. Wochenschr.* 1910, page 1530.

⁴ G. O. Gaebel. Salvarsan in the Forensic Detection of Arsenic. *Arch. d. Pharm.* 249 (1911), 49.

distilling in a stream of hydrochloric acid with addition of ferrous chloride, Gaebel¹ in two determinations obtained results for arsenic agreeing very well with each other but deviating considerably from calculated values. Instead of 33.6 per cent of arsenic, he found only 29 and 29.5 per cent. Moreover after destroying the organic salvarsan complex in a Kjeldahl flask with nitric and sulphuric acids, he again found only 29.3 per cent. of arsenic.

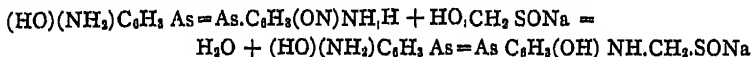
Neo-Salvarsan and Sodium-Salvarsan

Neo-salvarsan is a yellowish powder of characteristic odor dissolving easily in water with neutral reaction. It is a salvarsan derivative ready for use by simple solution in water and requiring no alkalization nor other treatment as the old salvarsan does. At the same time the activity of neo-salvarsan in correspondingly higher doses is the same as that of salvarsan. Neo-salvarsan is very sensitive to atmospheric air, quickly becoming highly toxic in contact with oxygen. For this reason this preparation is supplied in glass ampullae filled with an indifferent gas and sealed by fusion. Thus protected from oxidative processes this preparation keeps indefinitely. It contains about 20 per cent of arsenic. In addition to the active arsenical compound, it also contains inorganic salts, especially a large quantity of common salt. With regard to arsenic-content

- 1 gram of Salvarsan corresponds to ○ 15 gram of Neo-salvarsan = Dose I
- 2 gram of Salvarsan corresponds to ○ 30 gram of Neo-salvarsan = Dose II
- 3 gram of Salvarsan corresponds to ○ 45 gram of Neo-salvarsan = Dose III
- 4 gram of Salvarsan corresponds to ○ 60 gram of Neo-salvarsan = Dose IV

Only in special cases that are exceptional are individual doses larger than ○ 6 gram of neo-salvarsan given to men, or ○ 45 gram to women. Neo-salvarsan solutions according to concentration are prepared with sterilized distilled water, or with diluted salt solution (○ 4 per cent at highest), at blood temperature (about 37°). Solutions having too high salt-content are hypertonic and easily cause turbidity. They should not be heated because neo-salvarsan is decomposed. Neo-salvarsan solutions should be used as soon as prepared, since they are oxidized even more easily than solutions of old salvarsan. Neo-salvarsan is applied intravenously, in rare cases intramuscularly.

Preparation—Formaldehyde-sulphoxylic acid, or methane-sulphinic acid, is allowed to act upon meta-diamino-para-dioxy-arseno-benzene, the mother-substance of salvarsan, and the condensation-product converted into its sodium salt. This is neo-salvarsan. The entire chemical process should be carried out with utmost care and with regard for certain precautions, otherwise substances of very high toxicity may be formed (Report from Höchst Color Works).



So neo-salvarsan may be designated as dioxy-diamino-arseno-benzene sodium mono-methane-sulphinate.

Sodium-salvarsan is a fine, golden-yellow powder of characteristic odor easily soluble in water with alkaline reaction. In atmospheric air it undergoes pro-

¹Loc. cit.

found chemical change attended with very marked increase in toxicity. As a result the preparation takes on a darker or brown color and becomes insoluble in water (Höchst Report). To protect salvarsan-sodium from action of light, it should be filled in glass ampullae *in vacuo*, or sealed. Its arsenic-content like that of neo-salvarsan is about 20 per cent. With regard to arsenic-content

0.1 gram of Salvarsan corresponds to 0.15 gram of Salvarsan-sodium = Dose I
 0.3 gram of Salvarsan corresponds to 0.45 gram of Salvarsan-sodium = Dose III
 0.5 gram of Salvarsan corresponds to 0.75 gram of Salvarsan-sodium = Dose V
 0.6 gram of Salvarsan corresponds to 0.90 gram of Salvarsan-sodium = Dose VI

In corresponding doses the potency of salvarsan-sodium is the same as that of salvarsan. Primarily salvarsan-sodium is employed intravenously but, if a careful technique is observed, it may also be injected intramuscularly or subcutaneously. It is further used rectally and in very small doses intralumbally.

Preparation.—Salvarsan as a diacid phenol is converted by sodium hydroxide into a disodium salt that may be isolated by means of a suitable precipitating agent. The entire procedure as in the case of neo-salvarsan should be carried out with utmost care and with regard for certain precautions, otherwise substances of very high toxicity may be formed.

Colorimetric Estimation of Neo-Salvarsan and Sodium-Salvarsan in Urine

(Autenreith and Taege¹)

As a di-primary aromatic amine salvarsan and derivatives in therapeutic use may be diazotized by nitrous acid. The diazonium salts formed may then be coupled in alkaline solution with monatomic and polyatomic phenols and thereby converted into azo-dyestuffs. Of the phenols, resorcinol is particularly well adapted for this purpose. Abelin has also recommended it for the qualitative detection of salvarsan in urine. Phloroglucinol is also suitable and has an added advantage over resorcinol that its alkaline solution is more stable and may be kept even for several weeks in dark bottles. Since, however, the phloroglucinol test is not more delicate, on account of the higher cost of the reagent the authors in their experiments have used resorcinol which may be purchased in any drug-store and is readily obtained pure by distillation (Bpt. 276°).

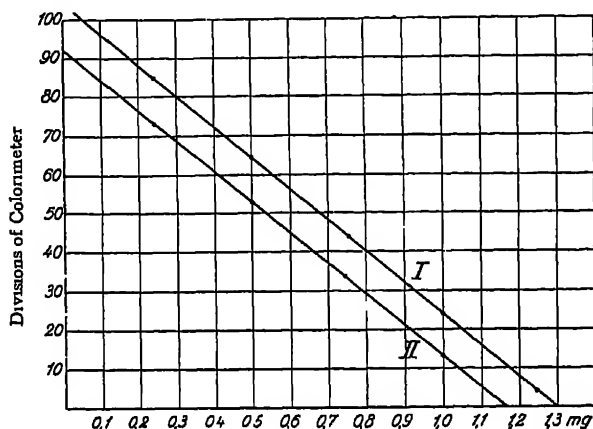
Calibration of the Comparison Wedge

As in all colorimetric determinations made with the Autenreith-Koenigsberger colorimeter, a comparison-wedge filled with an artificial, colored solution that can be kept without change is required. Such a solution having the same shade as that of the solution under examination is easily prepared by mixing solutions of cobaltous nitrate, ferric salt, copper sulphate and chrome alum. Such a wedge must first be calibrated. Dissolve 0.1 gram of neo-salvarsan in 200 cc. of water that has been previously boiled and cooled. To prevent oxidation, pass hydrogen through the solution at the same time. Also after diazotization excess of nitrous acid should be removed at once with urea, otherwise pure shades of color cannot be obtained with the resorcinol solution. With a capillary pipette

¹W. Autenreith and H. Taege. Elimination and Estimation of Salvarsan in Urine. München mediz. Wochenschr. 1922, page 1479.

exactly measure different quantities of the neo-salvarsan solution, that is, 0.5–2.5 cc. Each time deliver the solution into a small graduate well-cooled with ice, dilute to 5 cc with water, acidify with 3–4 drops of dilute hydrochloric acid, and finally add 4–5 drops of 0.5 per cent sodium nitrite solution. After 1 minute fill to the 10 cc mark with saturated urea solution, shake and at once return the graduate to the ice. Shaking from time to time soon decomposes excess of nitrous acid and a drop of the diazonium solution then colors starch-iodide paper neither blue nor violet. Then add 5 cc of a mixture, containing 5 cc of 6 per cent aqueous resorcinol solution and 3 cc of saturated sodium carbonate solution (about 20 per cent), previously prepared and cooled with ice. Shake thoroughly, return to the ice, and after 1–2 minutes repeatedly standardize against the same color strength. (For the full description of the colorimeter, see page 468.) In the calibration of a comparison-wedge the following correlated values were obtained:

Neo-salvarsan solution	0.5 cc	1 cc	1.5 cc	2 cc
Water	4.5 cc.	4 cc	3.5 cc	3 cc
Neo-salvarsan	0.25 mg	0.5 mg	0.75 mg	1 mg
Reading for equal color strength	83/84	62/63	43/44	23/24



Neo-salvarsan—Curve I—in 15 cc of color-solution or 5 cc of urine
Sodium-salvarsan—Curve II—in 15 cc of color-solution or 5 cc of urine

FIG. 28

These values are the basis of the above calibration curve of the comparison-wedge. In the same manner the calibration of the comparison-wedge for sodium-salvarsan may be made by dissolving 0.1 gram of this substance in 200 cc of water.

To estimate these substances in urine, the procedure is the same as that above for the calibration of the wedge, except that 5 cc of urine are measured instead of neo-salvarsan or sodium-salvarsan. By setting the color solutions in ice they may be kept about 5 minutes. Then a perceptible brown color appears. This may be due to resorcinol, for it does not appear when phloroglucinol is used.

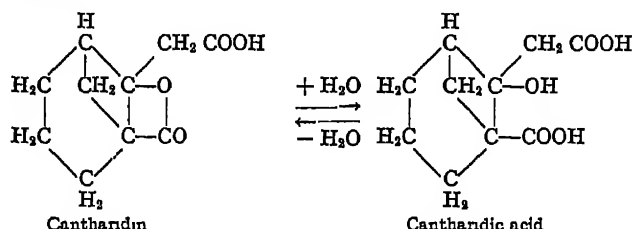
If possible, neo-salvarsan or sodium-salvarsan should be determined in freshly voided urine, since the content of these compounds may rapidly diminish on account of the ease with which they are oxidized even in atmospheric oxygen. In one instance a content of 18.7 mg of neo-salvarsan in a urine that stood in the air for a day dropped to 11.4 mg. On the basis of a long series of experiments the statement may be made that the quantity of salvarsan derivatives eliminated as such through the kidneys is exceedingly small as compared with that introduced intravenously. After intravenous injection the total quantity of neo-salvarsan, or sodium-salvarsan, eliminated through the kidneys ranges from 0.45–0.6 gram within a limit of 10–27 mg. In fact after the first 2 hours 5.2–10 mg are eliminated, in the third and fourth hour the quantity of salvarsan eliminated usually drops considerably, and 6 hours after injection hardly a trace of salvarsan can be found in the urine. Expressed in percentages these quantities are always small, usually less than 8 per cent of the salvarsan being eliminated in the urine. After injection of 0.6 gram = 600 mg of neo-salvarsan, 29 mg were eliminated in the urine within 4 hours, that is, not more than 4.6 per cent of the quantity of salvarsan introduced.

CANTHARIDIN

Cantharidin, $C_{10}H_{12}O_4$, the active vesicating principle of Spanish fly (*Lytta vesicatoria*), is present to the extent of 0.8–1 per cent. Cantharidin forms colorless, shining, neutral, rhombic leaflets, melting at 218° and subliming at higher temperature in white needles. Boiled with water and also with alcohol, cantharidin is volatile with water or alcohol vapor. It is almost insoluble even in boiling water. Acids, such as tartaric acid, increase its solubility in water, though cantharidin is not a base. It dissolves with difficulty in cold ethyl alcohol (0.03 : 100 at 18°) and in ether (0.011 : 100). Chloroform (1.52 : 100), acetone and acetic ether are its best solvents. It is practically insoluble in petroleum benzene.

Constitution.—Warmed with potassium or sodium hydroxide solution, cantharidin dissolves forming salts of cantharidic acid unknown in the free state. On the other hand, if solutions of these alkali salts are acidified and warmed, cantharidic acid at first set free loses water within the molecule and passes into its inner anhydride, cantharidin, which then separates. Potassium cantharidate, $K_2C_{10}H_{10}O_6 \cdot 2H_2O$, and sodium cantharidate, $Na_2C_{10}H_{10}O_6 \cdot 2H_2O$, are well crystallized salts. Cantharidin heated with hydriodic acid at 100° , or with chlorosulphonic acid, $ClSO_3OH$, at 0° , changes into the isomeric cantharic acid, $C_{10}H_{12}O_4$, which is crystalline and a strong monobasic acid. This acid is not a vesicant. Cantharic acid differs from cantharidin in being water-soluble. Acetyl chloride causes cantharic acid to undergo rearrangement and form the isomeric isocantharidin. The close relationship between cantharidin and o-xylene is shown by the fact that cantharic acid heated with caustic lime gives a dihydro-o-xylene, C_8H_{12} , the so-called cantharene, and cantharidin under the same conditions gives

with phosphorus pentachloride o-xylene Upon the basis of this chemical behavior H Meyer has assigned the following structural formulae to cantharidin and cantharidic acid



Cantharidin according to H Meyer is a β -lactone and at the same time a mono-basic acid. If the lactone ring is broken by caustic alkalis, the well crystallized alkali salts of dibasic cantharidic acid are formed. If these alkali salts are acidified with hydrochloric or sulphuric acid, at first free cantharidic acid is formed but this at once passes into cantharidin, its inner anhydride.

Toxic Action.—Cantharidin produces severe, local inflammation at the site of application, upon the outer skin or in the intestinal tract, at a distance it causes inflammation of the ureters and genital organs. Cantharidin acts upon the skin as a vesicant. In addition, a striking leucocytosis appears, as well as severe, acute inflammation of the kidneys, cantharidin nephritis. Although scarcely soluble in water, cantharidin is absorbed from the stomach as well as from the skin when intact. Before being absorbed from the latter, it causes inflammation and produces blisters.

Elimination in the urine of cantharidin as a salt of cantharidic acid starts in very soon. It also takes place through the intestinal glands. Consequently a secondary effect is intestinal irritation, even when the poison has not been taken by the mouth.

Cantharidin is a very powerful poison, for even 10 mg. may have a highly toxic action. As a rule death ensues after 1–5 days. In case of recovery frequently chronic inflammation of the kidneys persists.

Detection of Cantharidin in Organs, Blood and Urine

Render the material, stomach and intestines with contents, vomitus, blood, parts of liver, kidneys, brain, etc., strongly alkaline with dilute potassium hydroxide solution (1 gram of KOH in 15 cc of water) and then warm the mixture until thoroughly uniform. If present, cantharidin passes into solution as potassium cantharidate. The mixture should show an alkaline reaction, otherwise more potassium hydroxide solution must be added. When cold, shake with chloroform to remove unsaponified fat and other impurities. Draw off the aqueous alkaline solution and acidify with dilute sulphuric acid. Add 5 times the volume of alcohol and reflux for 30 minutes to convert cantharidic acid into cantharidin. Filter

when cold, distill¹ alcohol from the filtrate, and extract the unfiltered residue with chloroform. Evaporate the filtered chloroform extract, or distil the solvent, and use a portion of the residue for the physiological test. If this residue does not contain sufficient fat, dissolve in a few drops of hot almond oil. Bind a cloth saturated with this solution upon the upper arm or breast by adhesive tape. Cantharidin reddens the skin, sometimes raising pustules and blisters. Even 0.14 mg. is said to produce blisters. Salts of cantharidic acid also have a vesicating action.

If the physiological test is distinct or even strongly positive, J. Gadamer recommends attempting the preparation of pure cantharidin by the following method. Triturate the remainder of the residue from the above chloroform extract with petroleum ether, which dissolves no cantharidin, and then with a little cold alcohol, which dissolves scarcely any. What remains undissolved is recrystallized from acetic ether. The residue freed from fat by petroleum ether may be converted by warming with as little potassium hydroxide solution as possible into potassium cantharidate which owing to ready diffusibility can easily be purified by dialysis. The liquid outside the dialyzer may be treated with a little sulphuric acid and boiled for a very short time. This will yield almost pure cantharidin to chloroform. To recognize cantharidin, use may be made of the melting-point 218° , insolubility in water, solubility in potassium and sodium hydroxide solution, especially in presence of alcohol.

To examine urine or other liquids for cantharidin, acidify with dilute sulphuric acid and extract with chloroform which dissolves cantharidin. If urine contains albumin, first precipitate that by alcohol.

Notes.—Detection of cantharidin to be positive and free from objection cannot depend upon the physiological test alone, for pepper and paprika contain substances producing physiological results similar to those given by cantharidin. The article upon this matter in J. Gadamer's "Chemical Toxicology" (1911) should be consulted.

ARTIFICIAL HYPNOTICS AND SEDATIVES

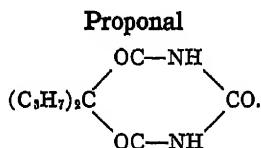
In his "Textbook of Pharmacology" (1918), Tappeiner divides hypnotics into two groups. The first embraces alkaloids that have

¹ Since cantharidin is volatile with the vapor of alcohol, evaporate the distillate after rendering it alkaline with a few drops of sodium hydroxide solution. Then acidify the residue with dilute sulphuric acid, heat to boiling, and extract with chloroform any cantharidin that may be present.

hypnotic properties, the second hypnotics of the aliphatic series. The former may also be designated as natural, the latter as artificial hypnotics. With few exceptions, artificial hypnotics belong to the aliphatic series. The author believes that a classification of artificial hypnotics suggested by Nolte¹ deserves consideration, for it arranges them according to chemical constitution in one of the following four sub-groups:

1. Derivatives of alcohol and related compounds.
2. Hypnotics belonging to the urea group.
3. Sulphonals that are derivatives of mercaptans.
4. Hypnotics that cannot be classified in the preceding groups.

A fifth group made up of combinations and transitions from one into the other groups might be added. The hypnotic effect of an agent, according to Bürgi, may be increased by combining two narcotics not belonging to the same group. In this case not only is the action of the two components additive but sometimes potency is increased. From this view-point has come into existence codeonal, a mixture of 2 parts of veronal-codeine (codeine diethyl-barbiturate) and 15 parts of medinal. This combination has proved of value in insomnia due to bronchitis. In general a hypnotic should act quickly and produce an effect of sufficient duration. Substances readily soluble in water, that are uniformly distributed in the stomach and absorbed in the intestines, will best meet these conditions. But above everything else hypnotics should not produce dangerous secondary effects upon circulation, respiration and metabolism. To render soluble various hypnotics like veronal and luminal, that dissolve in water with difficulty, especially for injection, convert them into sodium salts.



Proponal, dipropyl-barbituric acid, or dipropyl-malonyl-urea, belongs to the same class of compounds as veronal (see page 137). It is a white, crystalline powder, melting at 145° and having a faintly bitter taste. In cold water it dissolves with great difficulty, more

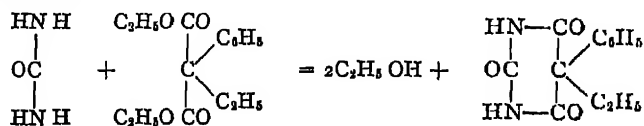
¹ E. Nolte: *Newer Hypnotics, Constitution and Action*. *Zentralblatt für Pharmazie* 17 (1921), 336

easily in hot, and readily in alcohol, ether, chloroform, and aqueous solutions of caustic alkalis. Acidification of alkaline solutions not too dilute again precipitates propanal

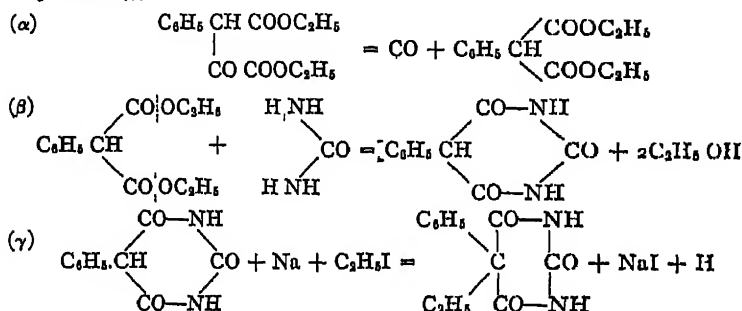
Physiological Properties.—Dialkyl-barbituric acids like disulphones show a relationship between chemical structure and physiological action. The dimethyl-compound has hardly any soporific action, the diethyl-compound, veronal, very marked action, and the dipropyl-compound, propanal, maximum action. The relative doses 0.5 gram of veronal = 0.25–0.3 gram of propanal about correspond to the difference in action. In therapeutic doses propanal produces sleep within 15–40 minutes. On the average, duration of sleep is 7 hours. In addition to being a hypnotic, propanal acts distinctly in allaying pain. Days after its use a feeling of exhaustion, stupor, vertigo and nausea may appear. Among secondary effects may be mentioned decrease in blood-pressure, on the other hand, accelerated heart action and increase of blood-pressure.

Luminal

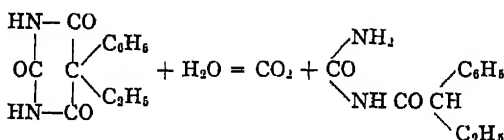
Luminal, phenyl-ethyl-malonyl-urea, or CC-phenyl-ethyl-barbituric acid, is formed by the action of urea upon CC-phenyl-ethyl-malonic ester:



Preparation.—Phenyl-oxalyl-acetic ester loses carbon monoxide on distillation and is converted into monophenyl-malonyl-diethyl ester (α) which with urea forms phenyl-barbituric acid (β) and then luminal on ethylation with sodium and ethyl iodide (γ)



From hot water luminal crystallizes in white, inodorous leaflets melting at 170–171° and having a faintly bitter taste. It is almost insoluble in cold water but freely soluble in organic solvents and dilute aqueous alkaline solutions. By continued action of alkalis luminal is decomposed into carbon dioxide and phenyl ethyl-acetyl-urea



Action of Luminal.—Luminal has a powerful hypnotic action. Koenig¹ has obtained very good results with luminal in tablet form and subcutaneously in hundreds of cases as a hypnotic and sedative, especially with women, and has observed exanthema in only two cases. Others have also recommended luminal as a good sedative and hypnotic and van Reysschoot (Bulletin de la Société de Médecine mentale de Belgique 1913) has pronounced it the best of our drugs as an anti-epileptic. Luminal, according to von Klebelsberg,² has a very favorable action upon epilepsy. Apparently it may even cure the disease in cases where bromine proved ineffective. Secondary effects, though not observed very frequently, call for special care on account of their severity.

Secondary Effects.—After administration of 4 luminal tablets of 0.3 gram each, Müller³ observed severe vomiting, disturbances of speech, unsteady gait, headache, vertigo, and disturbances of vision in conjunction with narcotic action. Itching exanthema of the skin appeared 8 days after this dose of luminal. Haug⁴ previously reported two cases of luminal poisoning. In one case, following administration of 0.1 gram of luminal 3 times daily, suddenly scarlet exanthema with high fever and bloody, mucous diarrhoea appeared 4 weeks after the beginning of treatment. After stopping the drug recovery followed. In the second case high fever and scarlet exanthema together with copious mucous diarrhoea without blood, also albuminuria and severe interference with the sensorium, appeared after using luminal for 11 days. In this case too these effects disappeared when use of luminal was stopped.

Detection of Luminal.—Add luminal to urine and then examine this artificial luminal-urine by the Stas-Otto process. Evaporate the urine after acidification with tartaric acid. Take up the residue in alcohol, filter and evaporate the filtrate. Dissolve the residue in water and extract with ether. By this method 75 per cent. of the luminal taken may be recovered in the urine. Following administration, however, of 0.3–0.6 gram of luminal, none could be found in the urine (Nolte⁵). Consequently the supposition is that even

¹ H. Koenig. Critical Notes upon Luminal. Berliner klinische Wochenschrift 1912, No. 40.

² v. Klebelsberg. Experiences with Luminal. Psychiatr.-Neurol. Wochenschrift 15 (1913), No. 34.

³ W. Müller. Secondary Effects from Luminal. Therapeutische Monatshefte 34 (1920), 79.

⁴ W. Haug. Two Cases of Luminal Poisoning. Münchener medizinische Wochenschrift 66 (1919), 1494.

⁵ E. Nolte. Pharmaz. Zeitung 65 (1920), 320.

after large doses, or protracted use of luminal, it cannot be detected either in parts of the cadaver or in urine. Since presence of a phenyl in place of an ethyl group is the only difference between luminal and veronal, the former appears to be the deciding factor in bringing about the complete decomposition of luminal in the animal organism. Before testing for luminal, recrystallize the given substance from hot water with use of animal charcoal and then take its melting-point. Use may also be made of its solubility in dilute alkalis, sodium carbonate solution and ammonia and of the fact that it is reprecipitated upon acidifying these solutions, if not too dilute.

Sodium-Veronal (also called **Medinal**).—This substance is the mono-sodium salt of diethyl-barbituric acid. It is a white, crystalline powder, having rather a bitter and somewhat caustic taste and dissolving in 5 parts of cold water. Sodium-veronal appears in commerce as the substance itself and as 0.5 gram tablets.

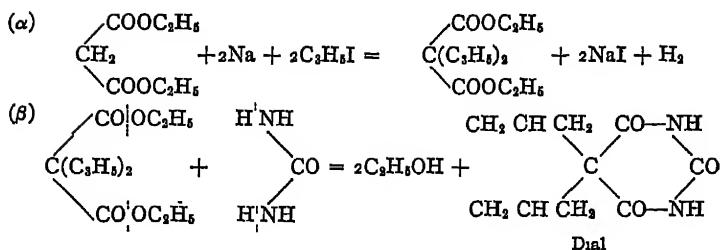
Physiological Action.—Therapeutic doses of 0.5 gram of veronal taken at a time produce in man within 0.5–0.75 of an hour sleep that as a rule is like physiological sleep and lasts 7–8 hours. Appearance of action depends upon the time it takes veronal to reach the intestinal tract where it is absorbed as alkali salt. When in solution, especially if the stomach is empty, veronal acts before it leaves the stomach, that is, more rapidly than when undissolved. On this account sodium-veronal is to be preferred to veronal itself, as it is freely soluble in water and consequently reaches the intestine quickly enough even though it is used undissolved.

Sodium-Luminal.—This compound is the mono-sodium salt of phenyl-ethyl-barbituric acid and is obtained by dissolving luminal (1 mol.) in sodium hydroxide solution (1 mol. NaOH) and evaporating the solution to dryness. It is a white, crystalline, hygroscopic powder freely soluble in water and especially adapted for subcutaneous use. Upon long standing, or after prolonged boiling of solutions of sodium-luminal, carbon dioxide is set free and phenyl-ethyl-acetyl-urea having only a feeble hypnotic action is precipitated. For this reason solutions of this compound should not be kept too long.

DIAL

Dial or diallyl-barbituric acid, that is, diallyl-malonyl-urea, is a hypnotic belonging like veronal, propional and luminal to the urea-group.

Preparation.—The diethyl ester of diallyl-malonic acid is obtained by the action of sodium and allyl iodide upon diethyl-malonate (α). This product treated with urea gives dial (β):



Physiological Action.—Injections of dial, according to Castaldi,¹ produce sleep rather rapidly in frogs and rabbits, as this drug does in man when taken by the mouth. A relatively small dose of dial, as compared with that of other hypnotics of the urea-group, produces an effect. This is ascribed to the presence of allyl-groups. In rabbits, following intravenous administration, an accelerating effect upon the motor heart-ganglia appears. On account of its action upon the heart and the ease with which man becomes habituated to dial, this compound has few advantages over other malonyl-urea derivatives having similar properties. Occasionally headache and vertigo appear after use of dial.

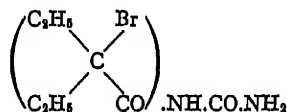
Didial is a combination of dial and dionine, or ethyl-morphine. It is used especially to produce twilight sleep. According to Bürgi, hypnotic action may be increased by combining two narcotics not belonging to the same group. In this case not only is an added effect from the two components obtained but sometimes action is more potent.

ORGANIC HYPNOTICS CONTAINING BROMINE

(Adaline, Bromural, Neuronal, Diogenal)

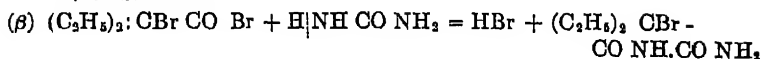
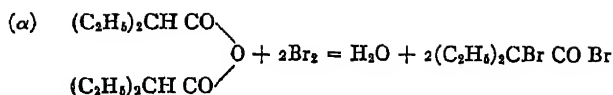
ADALINE

Adaline is the medico-pharmaceutical name given to bromo-diethyl-acetyl-urea:

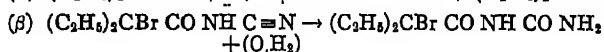
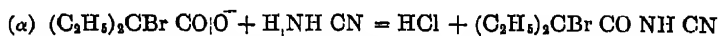


Preparation.—Bromo-diethyl-acetyl-bromide, formed by the action of bromine upon tetra-ethyl-acetic anhydride, is converted into adaline by urea:

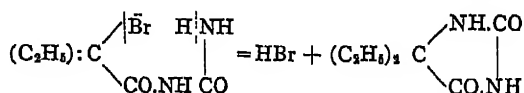
¹L. Castaldi. Pharmacological Properties of Diallyl-malonyl-urea. Arch. di Farmakol. speriment. 19 (1915), 289.



It may also be prepared by the addition of a molecule of water to bromo-diethyl-acetyl-cyanamide, formed by the action of cyanamide upon bromo-diethyl-acetyl chloride:



Adaline is a colorless, inodorous and nearly tasteless, crystalline powder melting, but not sharply, at 114–118°. In cold water it dissolves to the extent of only 0.05 per cent. but is more readily soluble hot; in alcohol, benzene and acetone it is freely soluble. When boiled with water, it is decomposed and also by sodium hydroxide solution. From warm alcohol with water it crystallizes in white needles melting at 117–118°. After heating with water, Br⁻-ion can be detected with silver nitrate. With aqueous potassium hydroxide solution it evolves ammonia; with alcoholic potassium hydroxide solution it gives ammonia, potassium bromide and potassium cyanide. Upon longer heating with water, but best with pyridine, apparently α -diethyl-hydantoin is formed, crystallizing from benzene in needles melting at 182–183° (Rosenmund and Herrmann¹)



Physiological Properties.—Adaline is a sedative having a feeble hypnotic action. Upon rabbits according to Filippi,² it has a poisonous action in doses of 0.7 gram per kilogram of body-weight, produces narcoses with 0.64 gram, and light but quite lasting sleep with 0.53 gram. It is said to have no injurious action upon the heart. Adaline does not pass into solution until it reaches the alkaline intestinal juice. Elimination of only a small part unchanged takes place through the urine. Most of it is present in the urine as a brominated acid, possibly as diethyl-bromo-acetic acid, $(C_2H_5)_2CBr \text{ COOH}(?)$. The acid isolated from urine melts at about 87°. Bromine in inorganic combination is split out from adaline

¹ K. W. Rosenmund and F. Herrmann: Contribution to the Knowledge of Adaline. *Berichte d. Deutsch. pharmaz. Ges.* 22 (1912), 86

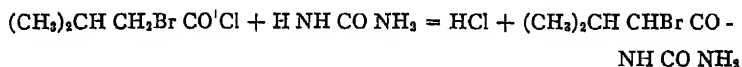
² E. Filippi: Pharmacological Properties of Adaline. *Arch. d. Farmakolog. sperim.* 12 (1911), 283

only after administration of toxic doses. According to Airila,¹ the lethal dose for rabbits is 0.7 gram per kilogram of body-weight.

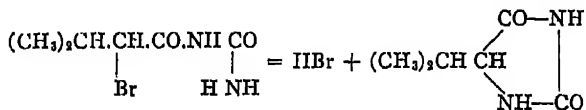
BROMURAL

Bromural is the commercial name of the hypnotic, α -bromo-isovaleryl-urea, $(\text{CH}_3)_2\text{CH}.\text{CHBr}.\text{CO}.\text{NH}.\text{CO}.\text{NH}_2$.

Preparation.—This compound may be prepared by the action of urea upon α -bromo-isovaleryl chloride or α -bromo-isovaleryl bromide:



Bromural crystallizes from toluene in leaflets melting at about 149° (Knoll). According to Zernik,² bromural, after repeated solution in a little warm alcohol and precipitation with water, forms white needles melting constant at 154° with previous softening. Though but very slightly soluble in cold water, it dissolves easily in hot water, alcohol, ether, and aqueous alkalis. At a higher temperature bromural can be sublimed. As an acid amide it can be hydrolyzed by acids and alkalis. Boiled with nitric acid, or 50 per cent sulphuric acid, bromural undergoes partial decomposition. The odor of valeric acid is apparent but bromine is not split out. Partial decomposition is also caused by boiling with 10 per cent sodium hydroxide solution, whereby ammonia is evolved and bromine is split out. If bromural (3 grams) is boiled under reflux for 3 hours with 0.5 N-alcoholic potassium hydroxide solution (40 cc.), a mixture of potassium bromide and cyanide separates. At the same time isopropyl-hydantoin is formed. This compound crystallizes from water in compact white crystals melting at $216\text{--}217^\circ$.



Isopropyl-hydantoin is very slightly soluble in cold water, more readily in hot water and alcohol, and only slightly in ether. Aqueous alkalis also dissolve it, causing the appearance of a faint red color and evolution of ammonia. Boiled with dilute sulphuric acid, it separates in only drops soluble in ether.

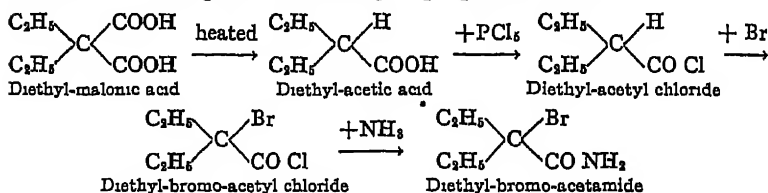
¹ Y. Airila: Experimental Investigations upon Bromural and Adaline. Skand. Archiv f. Physiologie 28 (1913), 193.

² F. Zernik: Bromural. Apotheker-Zeitung 22 (1907), 960.

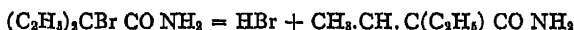
Physiological Action.—According to Airila (see Adaline), the two bromine-substituted acyl-ureas, bromural and adaline, have the same general action upon rabbits but that of adaline is somewhat stronger. The lethal dose of bromural for a rabbit is 10 gram per kilogram of body weight. In bromural poisoning the chief symptoms are: paresis¹ of the posterior extremities after 6–40 minutes, 20–50 minutes later, diminution of sensitiveness to pain and of the reflexes, increase of the secretory processes, tracheal rattling, trembling, decrease of respiration, lowering of temperature. After the lethal dose, death ensues in 5–24 hours; after smaller doses, recovery takes place in 5–36 hours. The main effect from bromural, as well as from adaline, is enlargement of the blood-vessels resulting in a marked lowering of blood-pressure. Frequency of breathing is strongly retarded due probably to the direct action of bromural upon the respiratory centre. On the contrary, bromural does not have an injurious action upon the heart.

NEURONAL

Diethyl-bromo-acetamide, $(C_2H_5)_2CBr.CO.NH_2$, is known as neuronal. The equations showing its preparation are:



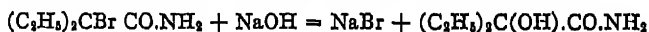
Neuronal is a white, crystalline powder melting at 66–67°, having an odor faintly suggesting camphor and a burning, slightly bitter taste. In cold water it dissolves 1:120 and is easily soluble in alcohol, ether, chloroform, and fatty oils. Neuronal in aqueous solution reacts acid and upon boiling is decomposed into hydrobromic acid and α -ethyl-crotonamide (Mannich and Zernik²):



When boiled with sodium hydroxide solution, neuronal is decomposed into hydrobromic acid, hydrocyanic acid and diethyl-ketone:



and partly into sodium bromide and diethyl-glycolamide:



In doses of 0.5–2 grams, neuronal acts as a hypnotic and sedative. At the present time fatal poisonings from neuronal are unknown.

¹ Paresis means a slight paralysis

² C. Mannich and F. Zernik. Contribution to the Knowledge of Neuronal. Archiv d. Pharmazie 246 (1908), 178.

Hydrocyanic acid is split off from neuronal even in the cold and with very dilute alkali. Apparently such a cleavage does not take place in the human organism. Following administration of neuronal, bromine can be detected in the urine even after 14 days and longer.

Detection of Neuronal

The following reactions may be used for the detection of neuronal (Zernik¹):

Like all organic substances containing bromine, neuronal imparts a blue-green color to a colorless Bunsen flame when burned upon a carefully oxidized copper wire.

If 0.2 gram of neuronal is boiled for a few minutes with 0.1 gram of mercuric oxide and 5 cc. of water, the hot filtered solution upon cooling deposits a white precipitate of mercury-neuronal. Addition of a few drops of potassium iodide solution causes the appearance of a light yellow, voluminous precipitate that gradually becomes crystalline and scarlet-red upon standing.

If 0.1 gram of neuronal is dissolved by boiling in 1 cc. of sodium hydroxide solution and 4 cc. of water, the odor of diethyl-ketone appears. Hydrocyanic acid may be detected in the solution by the Prussian blue reaction. After saturation with dilute sulphuric acid, hydrobromic acid may be detected with potassium permanganate solution and chloroform by the bromine coloration of the latter.

Distribution of Adaline, Bromural and Neuronal in the Organism

To establish distribution of a narcotic between the brain on the one hand and the rest of the body on the other, Gensler² took into consideration for the body only the protoplasm actually functioning and susceptible to narcosis. Consequently the weight of the skin, bones, contents of the gastro-intestinal tract and bladder was subtracted from the weight of the body. To determine hypnotic (adaline, bromural, neuronal) in the brain, the latter was first dried upon the water-bath, finely comminuted, mixed with 55 per cent of its weight of anhydrous disodium phosphate (Na_2HPO_4), completely dried in desiccator, and then extracted for 8 hours in a Soxhlet with ether. The solvent was distilled off and the residue, ashed after addition of a little potassium hydroxide, examined for bromine. The colorimetric method of estimating bromine (see page 208) may also be used. The relation between the total quantity of hypnotic absorbed and that

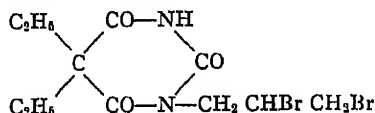
¹ F. Zernik. New Drugs for the Year 1904. Ber. d. Deutsch. pharm. Ges. 15 (1905), 6 and Apotheker-Zeitung 1904, 873.

² P. Gensler. Distribution of Neuronal, Bromural and Adaline in the Organism Archiv f. experim. Pathol. u. Therapie 79 (1915), 42.

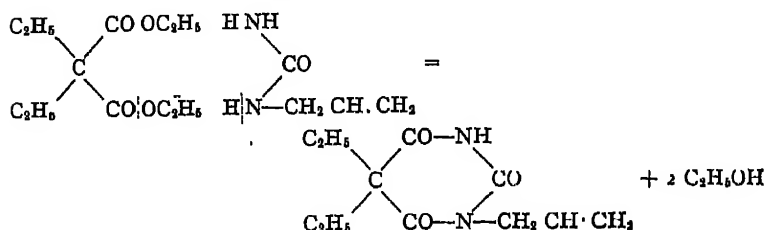
in the brain appears to be quite constant. In case of neuronal, the average percentage in the brain was 1.4 of that absorbed, bromural 1.7 per cent, and adaline 1.1 per cent. Deviations from these mean values are accounted for by different absolute weights of the brains. Although by far the greater part of the narcotic is distributed throughout the rest of the body and in the blood, the brain relatively has the greatest capacity of absorbing the hypnotics examined (adaline, bromural, neuronal). Upon the average 100 grams of brain contain almost twice as much of the given hypnotic as do 100 grams of all the rest of the protoplasm, blood included. The absorbed hypnotic in acting upon the brain undergoes no decomposition. To produce sleep of the same depth with the weaker hypnotic, more of it must be given and the brain contains a correspondingly greater fraction.

ADIOGENAL

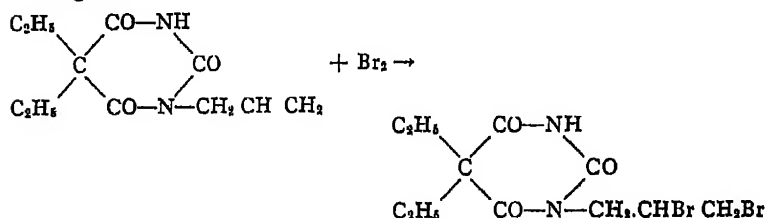
Diogenal is dibromo-propyl-diethyl-malonyl-urea, or dibromo-propyl-diethyl-barbituric acid



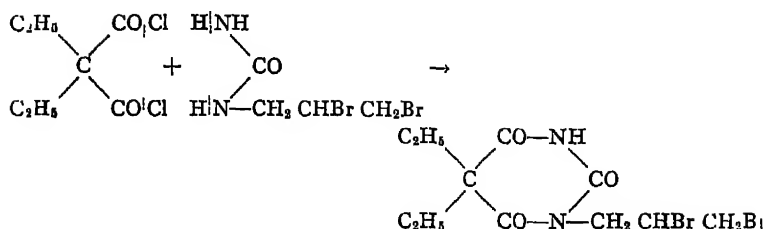
Preparation.—Mono-allyl-urea condensed with the diethyl-ester of diethyl-malonic acid gives mono-allyl-diethyl barbituric acid



When dissolved in glacial acetic acid, this compound takes up 2 atoms of bromine and forms dibromo-propyl-diethyl-barbituric acid = diogenal:



Diogenal is also formed by condensing dibromo-propyl-urea with diethyl-malonyl chloride:

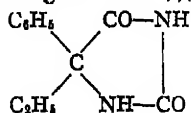


Diogenal appears as a white, crystalline powder, or in colorless needles, having a rather bitter taste and melting at $124-126^\circ$. It is obtained in the latter form by precipitating it from solution in hot toluene with petroleum ether, or by crystallization from cold alcohol. It is almost insoluble in water, readily soluble in alcohol and ether, and also in dilute sodium carbonate solution.

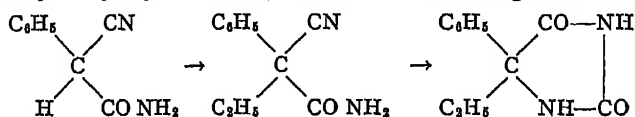
Pharmacological Properties—Diogenal contains the diethyl-barbituric acid radical, of so much value in medicine because of its narcotic action. In the side-chain attached to nitrogen is also halogen alkyl that manifests, especially in bromine derivatives, a powerful sedative action characteristic of organic bromine derivatives. Consequently diogenal closely resembles veronal in action. Introduction of the dibromo-propyl group into the veronal molecule produces the veronal action in milder form. The sedative character and influence of veronal, combined with the action of the bromine component, becomes more prominent, whereas the characteristic hypnotic effect is less marked. Diogenal is only slightly toxic in action. In case of warm-blooded animals its general action is analogous to that of veronal but considerably weaker. In a rabbit 0.25 gram of veronal taken internally as a rule produces sleep and 1 gram death; but 1 gram of diogenal produces sleep and not until more than 3 grams have been taken does deep coma sometimes ending in death appear. Experiments upon animals have also shown that more frequent administration of even larger doses of diogenal cause neither functional nor organic disturbance. Blood and urine remain normal as well as the pulse and blood-pressure. The bromine in diogenal is eliminated only gradually, a large part being retained. This presents the possibility of accumulation of bromine in the organism and of effects arising from the action of this halogen. Diogenal is stable in presence of dilute hydrochloric acid as it occurs in the gastric juice but slowly dissolves in the alkaline intestinal juice. Hardly any deleterious secondary effects have been observed even after prolonged use of diogenal. And up to the present time no indication has been given of cumulative action or chronic poisoning, even following use of diogenal for a month and longer.

NIRVANOL

Nirvanol is the name given to γ,γ -phenyl-ethyl-hydantoin,



Preparation.—Phenyl-ethyl-acetamide, formed by the action of an ethyl haloid upon phenyl-cyano-acetamide, is converted into γ,γ -phenyl-ethyl-hydantoin by means of hypohalogenites:



Nirvanol crystallizes in colorless, inodorous leaflets having hardly any taste. Its solubility in cold water is 1:1600; in hot water 1:110, in alcohol 1:20, and in ether 1:200. Like veronal and luminal it dissolves easily in sodium hydroxide solution forming a sodium salt. The hot saturated aqueous solution reddens litmus paper faintly. It melts at 199–200°. The sodium salt of nirvanol dissolves easily in water and its solution has a bitter-sweet taste.

Reactions for Detection of Nirvanol.—1. If 0.1 gram of nirvanol is heated with 0.5 gram of calcium oxide, the mass takes on a violet-red color, evolves ammonia, and develops the odor of propiophenone.

2. A solution of 0.1 gram of nirvanol in 2 cc. of sulphuric acid is colored red upon addition of 5 drops of sodium nitrite solution.

Therapeutic Use and Toxic Action of Nirvanol.—In hypnotic action nirvanol stands about midway between veronal and medinal. In cases of mild insomnia, especially in neuresthenics, the dose is 0.25–0.3 gram, in more stubborn cases 0.5–1.0 gram. As with veronal, sleep follows after about 2 hours. Medicinal doses do not cause weakening of the pulse, loss of appetite, headache or nausea (Tilling¹). Only gradual increase of lassitude indicates a cumulative effect of nirvanol. Following administration of nirvanol, no effect upon the centres controlling the heart and respiration is noticed. Nor is any evidence of kidney irritation given. Elimination of urine is normal and it is free from albumin and sugar. The body temperature is not lowered by nirvanol as it is in poisoning by veronal. On the contrary, children show several tenths of a degree increase of fever the same day they take the drug. But the fever drops the day after and shows a tendency to decrease further. The same thing occurs with luminal. Appearance of an exanthema presents an additional analogy to luminal. Curschmann² is probably the first to describe a severe case of nirvanol poisoning. In all the patient took 7 grams of nirvanol in 2.5 days. He was very sleepy, slept much both day and night but woke spontaneously between times, ate little, did not vomit, but was very thirsty. The buoyancy of his spirits and the absence of any unpleasant feeling of illness were quite noticeable. At the same time he was somewhat uncertain as to time and place of the most recent events and easily

¹ F. Tilling. Experiences with the New Hypnotic "Nirvanol." *Therapeutische Monatshefte* 32 (1918), 422.

² Curschmann. Therapeutic and Toxic Action of Nirvanol. *Therapeutische Monatshefte* 32 (1918), 53.

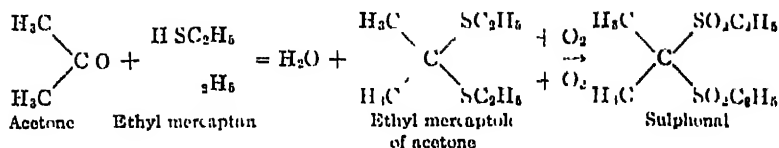
became delirious. He also complained of double vision. His pupils were very much contracted and reacted sluggishly. Respiration was regular, 14-18 to the minute, and pulse 76 to the minute, regular, quite full and firm. Temperature did not increase. Urine contained neither albumin nor sugar. During the entire period of intoxication vomiting did not occur. Curschmann emphasizes especially the fact that during this severe nirvanol poisoning lasting 2 5 days no serious disturbances of circulation and respiration appeared in spite of the large doses of the drug. Although Curschmann lists nirvanol with the more harmless hypnotics, such as adaline and bromural, and not among the "dangerous remedies," other clinicians advise caution because of severe secondary effects that may arise from nirvanol poisoning. Up to the present time have been observed facial oedema, increase of temperature and cutaneous eruptions, often accompanied by feelings of severe illness. After three doses of nirvanol of 0.5 gram each on three succeeding days, fever and appearance of exanthema were observed. Brüncke gives the following brief summary of his opinion of nirvanol as a hypnotic: "Nirvanol has certain advantages over other hypnotics but doses over 1.0 gram should not be given, and administration after 3-4 days should be stopped." Acetyl-nirvanol, recently recommended as a hypnotic, is said to be without the secondary effects of nirvanol.

SULPHONAL

Sulphonal, $(\text{CH}_3)_2\text{C}(\text{SO}_2\text{C}_2\text{H}_5)_2$, crystallizes in colorless, inodorous and tasteless prisms, melting at $125-126^\circ$ and distilling with slight decomposition at 300° . It dissolves at 15° in 425 parts of water, at 37° (blood temperature) in 220 parts, and at 100° in 8 parts. It is also soluble at 15° in 65 parts of alcohol (90 per cent.), in 80 parts of ether, and in 3.5 parts of chloroform. It is volatile with steam. At ordinary pressure it begins to evaporate at about 66° and the vapor has a peculiar odor (Falck¹). Solutions of sulphonal do not affect litmus paper. Especially characteristic of this compound is its great stability in presence of chemical agents. Halogens, halogen acids, alkaline hydroxides and carbonates, concentrated sulphuric and nitric acids are without action in the cold.

Preparation.—Condensation of ethyl mercaptan (2 molecules) with acetone (1 molecule) by means of dry hydrogen chloride gas, or concentrated sulphuric acid, results in formation of the ethyl-mercaptole of acetone. The latter, shaken with a saturated solution of potassium permanganate in presence of dilute sulphuric acid, undergoes oxidation with formation of dimethyl-methane-diethyl-sulphone=sulphonal:

¹ A. Falck. Contribution to the Knowledge of Sulphonal, Pharm Zentralbl 1919, No. 36



Detection of Sulphonal

Ether, or better chloroform, extracts sulphonal from acid, neutral and alkaline solutions. Test the residue left upon evaporating these solutions as follows:

1. **Melting-point Test.**—Absolutely pure sulphonal melts at 125–126°. Crystallization from boiling water with the use of a little bone-black easily gives a pure product. Mix the given crystals with what is known to be pure sulphonal. If the substance in question is sulphonal, this mixture should also melt at 125–126°.

2. **Reduction Test.**—Sulphonal heated in a test-tube with powdered wood charcoal gives the characteristic mercaptan odor.

3. **Detection of Sulphur.** (a) **With Sodium.** Fusion of sulphonal in a dry test-tube with a little metallic sodium gives sodium sulphide. Dissolve the cold melt cautiously (unaltered metallic sodium!) in a little water, filter and test the filtrate with sodium nitroprusside solution for sulphide (see page 305).

(b) **With Potassium Cyanide.**—Fuse 1 part of sulphonal with about 2 parts of pure potassium cyanide in a dry test-tube. Note the penetrating mercaptan odor. Potassium sulphocyanate is also a product of the reaction. An aqueous solution of the melt, acidified with dilute hydrochloric acid, becomes deep red with 1–2 drops of ferric chloride solution.

(c) **With Powdered Iron.**—Sulphonal heated with pure powdered iron free from sulphur gives a garlic-like odor. Add hydrochloric acid to the residue. Hydrogen sulphide evolved blackens lead acetate paper.

In suspected sulphonal poisoning, examine the contents of stomach and intestines, brain, parts of organs, and especially the urine. Even when parts of the cadaver have entered into an advanced stage of putrefaction, detection of sulphonal is possible, since this substance is very resistant to putrefaction.

Physiological Action.—Sulphonal in doses of 1–2 grams, especially when taken in powder form with enough of a warm liquid to bring most of it into solution, produces sleep in 1–3 hours. Because of its slight solubility sulphonal is slowly absorbed. Therefore its hypnotic action is less rapid than that of many of the

other hypnotics. Owing to conditions unfavorable to its decomposition and elimination, sulphonal is retained by the organism. Thus it produces cumulative effects. When administered for some time without interruption and in considerable doses, much of the drug may collect in the body. This also accounts for its prolonged action, for a single dose of sulphonal, or trional, frequently produces sleep on the second night. This delayed action proves that even after 24 hours these two hypnotics circulate in the body in active form. Consequently sulphonal and trional are indicated as hypnotics, especially in cases where prolonged action is desired. In the usual medicinal doses sulphonal has no injurious action upon the circulation of the blood, respiration and processes of digestion. After very large doses, and also after uninterrupted use of smaller quantities of sulphonal, or even trional, symptoms of poisoning appear. These become manifest from an action upon the digestive system, metabolism, and central nervous system. Symptoms of sulphonal and trional poisoning include stupor, ataxia, constipation, vomiting and gastric pains. Evidences of kidney irritation (albuminuria and nephritis) are also to be seen. In many cases, though not in all, it extends as far as formation of urinary porphyrin: Spectroscopically this behaves very much like haematoporphyrin from haemoglobin though it cannot be identical with that substance. The dark red to brown color of the urine is a frequent but by no means constant symptom of sulphonal and trional poisoning.


Detection of Sulphonal and Trional in Urine

Sulphonal occurs in the urine in detectable quantity only after rather large doses, especially following uninterrupted administration of the drug. Elimination of unaltered sulphonal in the urine continues for several days. For this reason it is sometimes possible to find sulphonal even in the organism, when the poisoning terminated fatally several days later. This is a significant fact because death as a rule takes place after the lapse of several days. A sulphonal urine is often colored dark red to garnet-brown due to a content of haematoporphyrin (?). But this decomposition product of blood-pigment appears in urine only succeeding severe poisoning by sulphonal, and even then its occurrence is rare.

To isolate sulphonal from urine, evaporate 1000 cc. or more to about one-tenth its volume, and extract several times with large quantities of ether. Pass the combined ether extracts, after they have settled in a dry flask for several hours, through a dry filter and distil. Evaporate the residue with 20-30 cc. of 10 per cent. sodium hydroxide solution to dryness upon the water-bath. This will remove coloring matter, extracted from urine by ether, but will not affect sulphonal. Extract sulphonal from the alkaline residue with ether. Evaporate the solvent and sulphonal will remain pure and almost colorless. Determine the melting-point of this residue and make the other tests for sulphonal.

Detection of Haematoporphyrin in Urine in Sulphonal Poisoning

Coloring matters have been observed in red, brownish red or cherry-red urines, probably identical with haematoporphyrin. Spectroscopic examination of such urine is made in the following manner. Add sodium hydroxide solution drop by drop to about 0.3 liter of urine, until the reaction is strongly alkaline, and then add a little barium chloride solution. Filter after a while and wash the precipi-

ate well Extract the precipitate upon the filter with hot alcohol containing a few drops of dilute sulphuric acid. Spectroscopic examination of this filtrate may be made direct with a Browning pocket spectroscope. Acid haematoporphyrin solutions are violet, when more concentrated, they have a cherry-red color and show the characteristic spectrum with two absorption-bands. If the acid, alcoholic solution is saturated with a few drops of ammonium or sodium hydroxide solution, the spectrum of alkaline haematoporphyrin solution with its four absorption-bands appears. Traces of haematoporphyrin very frequently appear in normal urine. See absorption-spectra upon page 328.

TRIONAL

Trional, methyl-ethyl-methane-diethyl-sulphone, $(C_2H_5)(CH_3)-C(SO_2C_2H_5)_2$, crystallizes in colorless, shining, inodorous plates melting at 76° . It is soluble in 320 parts of water, the solution having a bitter taste and without action upon litmus paper. In the former property it differs from sulphonal which is tasteless. Trional gives the sulphonal reactions. Trional is completely decomposed in the human organism and danger of cumulative action is much less than in the case of sulphonal. Moreover, haematoporphyrinuria has almost never been observed, even following considerable doses of trional and after uninterrupted use for weeks.

SANTONIN

Santonin occurs to the extent of 2-3 per cent. in the unexpanded flower-heads of *Artemisia maritima* (Flores Cinae), also falsely called worm-seed. The blossoms after opening contain no santonin. Flores Cinae and santonin find use in medicine as a remedy for round and other intestinal worms.

Santonin, $C_{18}H_{18}O_8$, crystallizes in colorless, inodorous, shining leaflets that have a bitter taste and melt at 170° . Light turns these crystals yellow. Santonin dissolves in 5000 parts of cold and 250 parts of boiling water, in 44 parts of alcohol; in 150 parts of ether; and in 4 parts of chloroform. All these solutions are neutral. Upon evaporation, an alcoholic solution of the yellow modification deposits white santonin. Santonin is the internal anhydride (lactone) of santonic acid, $C_{18}H_{20}O_4$, for caustic alkalis, as well as calcium and barium hydroxides, dissolve santonin forming salts of this acid.

Toxic Action.—In man the principal evidences of poisoning are nervous disturbances of the retina recognized by yellow or violet vision, usually associated with sparkling. Occasionally nervous disturbances of the olfactory and gustatory nerves also appear. Additional manifestations are headache, dizziness,

trembling, convulsions, unconsciousness, vomiting and diarrhoea, icterus and fever. Pronounced slowing of the pulse, dilatation of the pupils, yellow coloration of the conjunctiva and of the urine have been observed. Even 0.06 gram of santonin is said to have caused death. As a rule, however, much larger quantities are tolerated. Elimination of santonin from the body takes place rather slowly. For this reason it acts cumulatively. Consequently poisonings may occur as a result not only of wrong dosage but of too long administration of santonin.

Behavior in the Animal Organism.—Santonin seems to be incompletely absorbed in the organism. Jaffé¹ has administered quite large quantities of santonin to dogs and rabbits. He obtained a new substance, called α -oxysantonin ($C_{14}H_{18}O_4$), from the urine of the dog, amounting to 5–6 per cent of the santonin administered. He extracted with chloroform considerable quantities of unaltered santonin from the faeces of the dog. Rabbits usually can tolerate being fed with santonin for weeks, and α -oxysantonin is formed only in very small quantity. In the ether extract of rabbit's urine, Jaffé found a second santonin derivative, β -oxysantonin, isomeric with α -oxysantonin, with considerable unaltered santonin. In these experiments only about half the santonin administered was absorbed by the rabbit.

After administration of santonin, a red pigment called santonin-red appears in human urine. Even after medicinal doses santonin urine is red, or becomes at least scarlet-red to purple on addition of potassium or sodium hydroxide solution. Urine containing santonin also becomes carmine-red on addition of calcium hydroxide solution.

Detection of Santonin

Ether, benzene, or better chloroform, extract santonin only from neutral or acid solutions. The organic solvent fails to remove this compound from an alkaline solution, as it is then in the form of a santonate. Santonin is not an alkaloid and forms no precipitates with the general alkaloidal reagents but gives several more or less characteristic color reactions.

1. Alcoholic Potassium Hydroxide Test.—Pure, white santonin, heated with an alcoholic solution of potassium hydroxide, gives a fine carmine-red color that gradually changes to reddish yellow and finally fades entirely. In this test yellow santonin dissolves with a yellowish red color.

2. Sulphuric Acid-Ferric Chloride Test.—If powdered santonin (0.01 gram) is shaken with a cold mixture of 1 cc. of sulphuric acid and 1 cc. of water, no color appears. But if the mixture is then heated almost to boiling with addition of a few drops of ferric chloride solution, it takes on a violet color.

¹ M. Jaffé. *Oxy-Santonin and Its Formation in the Animal Body after Administration of Santonin*. *Zeitschr. f. physiol. Chem.* 22 (1896 to 1897), 337.

3. **Furfural-Sulphuric Acid Test.**—Mix 2–3 drops of alcoholic santonin solution with 1–2 drops of 2 per cent. alcoholic furfural solution and 2 cc. of pure concentrated sulphuric acid. Warm this mixture in a small porcelain dish upon the water-bath. A purple-red color appears and changes with continued heating to crimson-red, blue-violet and finally to dark blue (Thaeter¹). Should this delicate santonin test not appear at once, add a few drops more of furfural solution. The play of colors described appears quite distinct with only 0.1 mg of santonin. In absence of santonin, the furfural-sulphuric acid is colored first pale red, later an off-shade brown.

Only a few alkaloids and glucosides give distinct color reactions with furfural and sulphuric acid. Substances behaving similarly are veratrine, picrotoxin (violet) and piperine (green to blue-green, finally indigo-blue). The colors given by α - and β -naphthol with furfural and sulphuric acid are also characteristic.

4. **Potassium Cyanide Test.**—Heating together a mixture of santonin and powdered potassium cyanide produces a dark red mass dissolving in water, or in solutions of caustic alkalies, with a strong green fluorescence.

Special Examination for Santonin

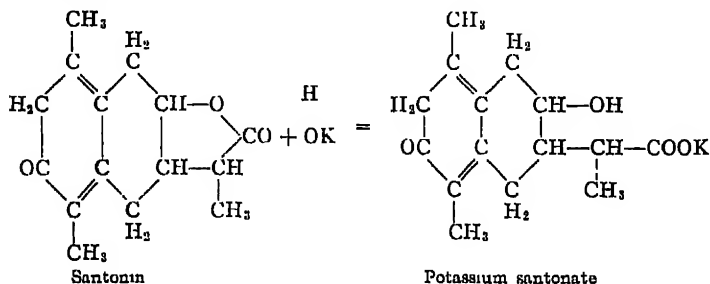
In suspected santonin poisoning, examine vomitus, contents of stomach and intestines, and also, if the poisoning is of very recent occurrence, organs and blood. Urine contains only decomposition products of santonin (see above).

Comminute the material as finely as possible and warm for several hours upon the water-bath with water containing sodium hydroxide, or according to Dragendorff with milk of lime, and then strain. To remove impurities from the liquid thus obtained, first shake with benzene, then acidify with hydrochloric acid, and again extract with benzene, or better with chloroform. Evaporation or distillation of solvent will leave santonin. The final solutions are purer, if the alkaline liquid is first freed from impurities by using about 3 times the volume of alcohol as a precipitant. Collect the precipitate upon paper, remove alcohol from the filtrate by distillation, and thoroughly extract the residual solution with benzene, when alkaline, and with chloroform, when acid. The latter then contains the santonin. Recrystallize crude santonin thus obtained from hot water, using animal charcoal to remove any color.

¹ K. Thaeter. Contributions to Forensic Chemistry. Archiv d. Pharmaz. 235 (1897), 401.

Chemistry of Santonin

Santonin is the internal anhydride (lactone) of santonic acid, $C_{15}H_{20}O_4$. Caustic alkalis, as well as calcium and barium hydroxides, dissolve santonin forming salts of this acid. In this case, as with all lactones, the lactone ring is broken as follows



A solution of a santonate, acidified with hydrochloric acid, first gives free santonic acid. To isolate this compound from the mixture, extract at once with ether. Otherwise, the acid loses 1 molecule of water upon standing and passes into its internal anhydride, santonin.

Santonin is also a ketone. As such it forms a hydrazone, $C_{15}H_{18}O_3 = N - NH \cdot C_6H_5$, with phenyl-hydrazine and an oxime, $C_{15}H_{18}O_3 = NOH$, with hydroxylamine.

According to the above structural formula, santonin is a derivative of hexahydro-dimethyl-naphthalene. Fused with potassium hydroxide, santonic acid gives hydrogen, propionic acid, and a naphthalene derivative, namely, dimethyl- β -naphthol.

Examination for Santonin,¹ Sulphonal and Trional

These potent drugs considered in this place do not find a place in the Stas-Otto process on account of their behavior toward cold tartaric acid solution and ether. The following method may be employed for their detection:

Extract the material, neutral or faintly acid with tartaric acid, under a reflux with boiling absolute alcohol. Filter hot and evaporate the filtrate to dryness upon the water-bath. Dissolve the residue in hot water. If the solution is colored, digest it for some time upon the water-bath with bone-black and stir frequently. Filter the hot solution. All of the above substances, if present in considerable quantity, crystallize in part as the solution cools. Thoroughly

¹ Because santonin manifests toward solvents a behavior similar to that of sulphonal and trional and is for that reason obtained by the same method of extraction, it is included in this place in connection with these hypnotics.

extract the filtrate and any crystals several times with chloroform. Pass the chloroform extract through a dry filter. The residue from chloroform may contain santonin, sulphonal and trional.

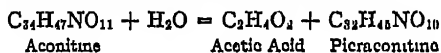
The chloroform residue may also contain those substances extracted in the Stas-Otto process from the acid solution by ether. Chloroform completely extracts substances such as colchicin, anti-pyrine, caffeine, acetanilide, phenacetine and salicylic acid. As a rule they are purer from this solvent than from ether. The chloroform residue may also contain the weak base narcotine

CERTAIN ALKALOIDS OF RARE OCCURRENCE

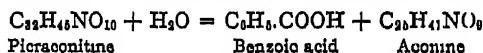
ACONITINE

Aconitine, $C_{34}H_{47}NO_{11}$, in combination with aconitic acid, occurs with other bases in *Aconitum napellus*, especially in the plant (0.3 per cent. total alkaloids) and in the tubers (about 0.6 per cent. total alkaloids). The tubers of other aconite species are said also to contain this alkaloid in smaller quantity. Pure aconitine forms colorless, tabular, rhombic crystals dissolving with difficulty in water but readily in alcohol, ether, chloroform and benzene. It is nearly insoluble in petroleum ether. Aqueous solutions of aconitine have a very sharp, persistently burning but not bitter taste. The very bitter taste of aconitine when not entirely pure is due to an admixture of an amorphous alkaloid known as picroaconitine.

Chemical Behavior.—Aconitine boiled with water, but more quickly in presence of mineral acid or alcoholic potassium hydroxide solution, undergoes hydrolysis first into acetic acid and the very bitter picroaconitine known to be benzoyl-aconine:



Upon further hydrolysis the latter yields benzoic acid and aconine:



These reactions show aconitine to be acetyl-benzoyl-aconine. Ordinary commercial preparations of aconitine contain the cleavage-products, picroaconitine and aconine, and to their presence may be attributed the bitter, sharp taste as well as the uncertain potency of commercial aconitine.

Reactions.—Even in very dilute solution aconitine is precipitated by such general alkaloidal reagents as phospho-molybdic acid, phos-

pho-tungstic acid, iodo-potassium iodide, potassium mercuric iodide, potassium bismuthous iodide, tannic acid, and gold chloride, whereas platinum chloride, mercuric chloride, and picric acid cause precipitation only in more concentrated solutions.

Concentrated sulphuric acid dissolves pure crystallized aconitine without color and concentrated nitric acid behaves in the same way. Careful evaporation of pure aconitine with officinal phosphoric acid produces either no result at all or only a very faint red color.

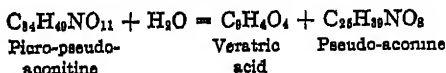
Physiological Behavior.—Aconitine should be classed with the most powerful poisons. At the outset it stimulates or irritates the terminations of the sensory motor or secretory nerves of the striated muscles, of the heart, and of the central nervous system (brain, spinal cord, emetic center, motor center of the intestines, center of respiration and of dilatation of the pupils). Then they are deadened or paralyzed so that death occurs as a result of paralysis of the heart or lungs. The symptoms of aconitine poisoning are burning in the throat, flow of saliva, vomiting, colic, diarrhoea, insensibility of the tongue, enlargement of the pupil, disturbance or loss of the sense of sight and hearing, marked slowing of the pulse, difficulty in breathing and convulsions. Aconitine is so easily absorbed even after external application that poisoning takes place quickly, in a few days or even hours. Elimination proceeds mainly through the urine which is especially adapted for the isolation of the poison.

Pseudo-Aconitine.—This alkaloid, $C_{36}H_{49}NO_{12}$, is found principally in the tubers of *Aconitum ferox*. They are said to contain no aconitine. On slow evaporation of an ether solution, or better of a mixture of ether and petroleum ether, pseudo-aconitine crystallizes in transparent needles or granular crystals. On rapid evaporation it is deposited as an amorphous or syrupy mass. Though slightly soluble in water, it is more easily soluble than aconitine in alcohol and ether. Solutions of pseudo-aconitine are dextro-rotatory; those of its salts laevo-rotatory.

Chemical Behavior.—Several hours boiling with water causes hydrolysis of pseudo-aconitine into acetic acid and picro-pseudo-aconitine:



The latter base is then further hydrolyzed by alcoholic potassium hydroxide solution into veratric acid and pseudo-aconine:



These reactions show that pseudo-aconitine is acetyl-veratryl-pseudo-aconine.

Reactions.—Pseudo-aconitine resembles aconitine in its behavior toward general alkaloidal reagents. Even in great dilution it is precipitated by potassium mercuric iodide, iodo-potassium iodide, tannic acid, and gold chloride, whereas platinic chloride causes precipitation only in more concentrated solutions. Pure pseudo-aconitine in presence of concentrated sulphuric and phosphoric acids gives no distinctive color reactions. According to Dragendorff, pseudo-aconitine isolated from the organs of animals poisoned by this alkaloid is said to behave like pure aconitine. By Vitali's test pseudo-aconitine behaves like atropine, for a small quantity of this alkaloid evaporated upon the water-bath with a few drops of fuming nitric acid leaves a yellow residue that takes on a fine purple-red color when moistened with alcoholic potassium hydroxide solution (1:10). Absolutely pure crystallized aconitine does not give this test.

In carrying out Jurgens test, Gadamer advises the following procedure. To test for aconitine or pseudo-aconitine in the alkaloidal residue obtained by evaporating the ether extract of the aqueous alkaline solution, dissolve in a drop of water containing acetic acid and add a small crystal of potassium iodide. When the latter has dissolved and the solution has evaporated spontaneously, dissolve the crystallized potassium iodide with a drop of water and quickly remove the solution. The iodide from pure aconitine appears in well-formed, tabular crystals of rhombic shape. Sometimes these crystals are cross-shaped and grow together obliquely, or lie together feather-like. Impure aconitine will not give this reaction but forms only amorphous masses. Pseudo-aconitine gives needle-shaped druses.

Fühner's Biological Detection of Aconitine.¹—To prevent decomposition of the exceedingly labile aconitine, extract the material at moderate heat with alcohol containing only a small quantity of tartaric acid. Remove alcohol from the filtered extract at as low a temperature as possible, best *in vacuo*. Filter the faintly acid, aqueous residue to get rid of fat and extract the filtrate with petroleum ether to remove impurities. Then render the aqueous solution alkaline with sodium carbonate (not with caustic alkali) and at once extract alkaloid with ether. Dissolve the residue left upon evaporating the ether extract in a little water containing acetic acid. Filter the solution, make alkaline with sodium carbonate, and again extract with ether. The residue from this ether extract may be used for Fühner's biological test for aconitine. Dissolve the

¹H. Fühner Toxicological Detection of Aconitine Arch f exper. Path. u Pharm 66 (1911), 179

residue in a little water containing acetic acid, add sodium carbonate to the filtered solution until neutral, dilute to 5 cc, and taste by dipping a strip of purest filter-paper in the solution and applying it for a short time to the tip of the tongue. After 5-10 minutes, if aconitine is present, a burning sensation is noticeable upon the tongue, similar to that often produced by too hot food. This irritation may persist for hours. This test, according to Fühner, is characteristic of aconitine. Using the same solution (5 cc), correspondingly diluted with Ringer's solution isotonic with the frog, Fühner also makes a test with isolated frog's heart. By this test 0.05 mg of aconitine may be detected with certainty. Of the three stages in the action of aconitine on the frog's heart, the second, that of heart convulsions or heart peristalsis, is specific for aconitine. Delphinine, an alkaloid of no forensic significance, alone resembles aconitine in its action upon the heart.

ARECOLINE

Areca-nuts, or betel-nuts, the seeds of *Areca Catechu*, contain several alkaloids. Areca-palms, originally indigenous to the Sunda islands, are now cultivated to a great extent in the warmer parts of the Indies as well as in the Philippines. Areca-nuts in their habitat are an important article of commerce, for mixed with lime and leaves of the betel pepper they are chewed by the natives. Occasionally they are also used in China and the Indies as a vermifuge. With the exception of choline, the following bases isolated by Jahns¹ are closely related: choline, guvacine ($C_8H_9NO_3$), arecaine ($C_7H_{11}NO_2, H_2O$), arecaine ($C_7H_{11}NO_2, H_2O$), arecoline ($C_8H_{13}NO_2$). Of these bases arecoline must be viewed as the physiologically active constituent and as the seat of the anthelmintic action of areca-nuts. The yield of arecoline is 0.07, at the highest 0.1 per cent, that of arecaine about 0.1 per cent (Jahns).

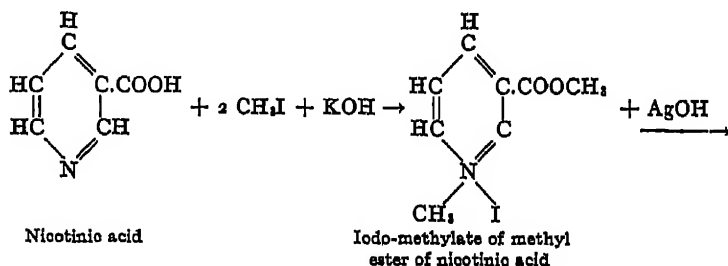
Arecoline is a colorless, oily liquid having a strong alkaline reaction and soluble in all proportions in water, alcohol, ether and chloroform. It boils at about 220° and is also volatile with steam. Most of its crystallizable salts are easily soluble and some are deliquescent. Of the simple salts arecoline hydrobromide ($C_8H_{13}NO_2 \cdot HBr$) used in medicine crystallizes best. Solutions of arecoline salts with potassium bismuthous iodide give a garnet-red precipitate consisting of microscopic crystals (delicate reaction), and a white precipitate with phosphomolybdic acid. If the solution is not too dilute, potassium mercuric iodide causes a precipitate of yellow, oily drops that become crystalline after several days, iodo-potassium iodide brown drops, and picric acid a resinous precipitate later changing to needles. Platinic chloride, mercuric chloride and tannic acid cause no precipitation. Arecoline gives no characteristic color reactions.

Physiological Action and Use.—Arecoline differs from the other areca-alkaloids in being strongly active. Its action is very much like that of pilocarpine. But its action upon the pupil of the eye, causing contraction, is even stronger than that of the latter and in this respect is closely related to the action of muscarine.

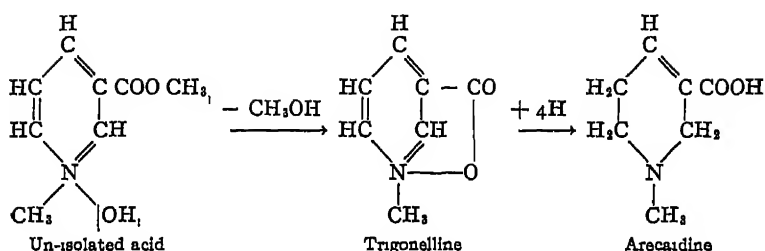
¹ E. Jahns: *The Alkaloids of Areca Nuts*. *Berichte d. Deutsch. chem. Ges.* 21 (1888), 3404; 23 (1890), 2972, 24 (1891), 2615.

Introduced into a cat's eye it may cause the pupil to disappear almost completely (Kobert). In its anthelmintic action it resembles pelletierine. Injected directly into the blood of dogs in almost lethal doses, it gives rise to convulsive attacks followed by paralysis of the central nervous system. In addition it increases mucous, salivary and sweat secretion. Arecoline in part is said to be eliminated undecomposed in the urine. The lethal dose of arecoline for a rabbit is 10 mg. On the other hand, rather small dogs have survived doses of 50-75 mg of the base. Arecoline resembles muscarine in its action upon the heart. Taken internally it causes ejection of intestinal parasites, especially tapeworm. Arecoline hydrobromide in doses of 0.004-0.006 gram is used for this purpose. A 1 per cent solution of this salt serves as a myotic, that is, an agent to cause contraction of the pupil in ophthalmology. In subcutaneous doses of 0.03-0.06 gram it is used in veterinary medicine as a remedy for colic in horses.

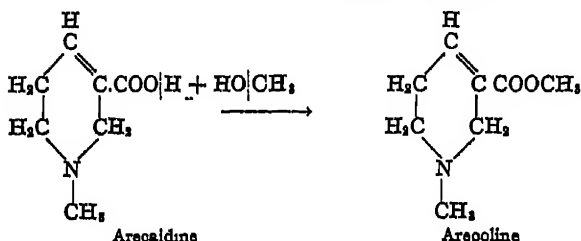
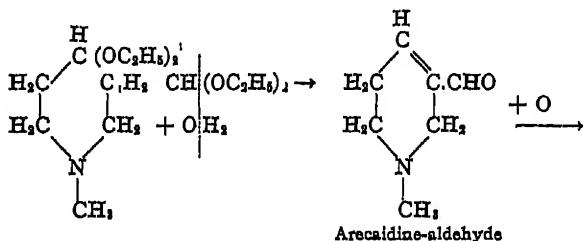
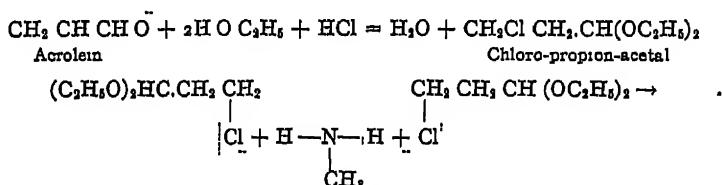
Constitution and Synthesis of Arecoline.—Heated with strong hydrochloric acid to about 150° , arecoline is decomposed into methyl chloride and arecaine. On the other hand, arecaine which has weak acid properties may be converted into arecoline by methylation, that is, by passing dry hydrochloric acid gas into a suspension of arecaine in methyl alcohol. Arecaine has been synthesized. This was accomplished by starting from nicotinic acid, or β -pyridine carboxylic acid, and passing through trigonelline¹ which also has been synthesized. Nicotinic acid is evaporated with potassium hydroxide (1 mol) and the dry residue heated to 150° with excess of methyl iodide. The iodomethylate of the methyl ester of nicotinic acid thus formed is digested with moist silver oxide. Careful evaporation of the filtrate leaves trigonelline, or the methyl-betaine of nicotinic acid, as colorless needles readily soluble in water and less so in alcohol. Trigonelline, or the iodomethylate of the methyl ester of nicotinic acid, is reduced when heated for some time with tin and hydrochloric acid. The product of reduction is then freed from tin by hydrogen sulphide. The resulting solution treated with silver oxide and then freed from silver by hydrogen sulphide yields methyl-hexahydro-nicotinic acid. This compound can be extracted with chloroform and is identical with dihydro-arecaine. A part insoluble in chloroform and formed at the same time, after treatment with absolute alcohol, leaves undissolved methyl-tetrahydro-nicotinic acid identical with arecaine:



¹ Trigonelline occurs to the extent of about 0.15 per cent. in the seeds of *Trigonella foenum graecum* and also in peas, hemp-seed and oats.



According to a synthesis of arecaidine discovered by Wohl and Johnson,¹ acrolein with alcohol is converted by means of hydrochloric acid gas into chloro-propion-acetal. The latter is heated to 125° with methylamine dissolved in benzene. Treatment of the base thus formed with fuming hydrochloric acid at 0° causes ring formation. The product is the hydrochloride of arecaidine-aldehyde which is converted by oxidation into arecaidine. Dry hydrochloric acid gas passed into a suspension of the latter in methyl alcohol converts it into arecoline.



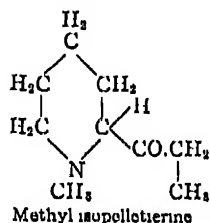
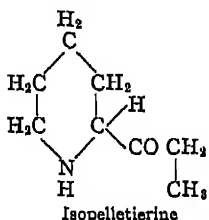
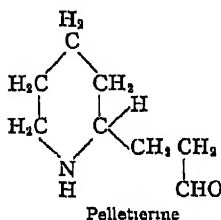
¹ A. Wohl and A. Johnson. Arecaidine and Arecoline. *Berichte d. Deutsch. chem. Ges.* 40 (1907), 4712.

Detection of Arecoline

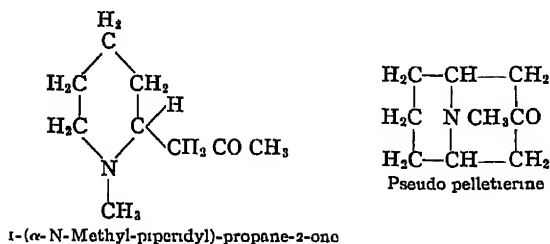
Volatility of free arecoline base with steam may be used to effect its separation. Its action in causing contraction of the pupil of the eye may be employed for its recognition, since it has no characteristic chemical reactions. To carry out this test, dilute the material with water, render alkaline with sodium hydroxide solution and distil. Exactly neutralize the distillate with dilute hydrochloric acid, evaporate to a few drops, and introduce the solution into a dog's eye, or better a cat's eye. Contraction of the pupil indicates presence of arecoline. An alternative procedure is to extract the distillate with ether, evaporate the ether extract, neutralize the residue with very dilute hydrochloric acid, and use this solution for tests upon the eye.

Alkaloids of Pomegranate Bark

The bark of the root and trunk of the pomegranate tree (*Punica granatum*) contains several alkaloids. Tanret (1878) was the first to isolate from this bark four volatile bases to which the names, pelletierine, isopelletierine, pseudo-pelletierine and methyl-pelletierine were given. As the fifth member of this group, Piccini added iso-methyl-pelletierine. In his description of the bases isolated and in the analyses of their salts Tanret characterized them as chemical individuals and gave them their true composition. He did not undertake to determine their chemical structure. Pseudo-pelletierine, $C_8H_{15}NO$, which crystallizes well and occurs in largest quantity in pomegranate bark, was recognized by Ciamician and Silber as a higher homologue of tropinone. This base was very useful to R. Willstätter in the synthesis of cyclo-octane derivatives. As a result of numerous recent investigations by Kurt Hess¹ and his collaborators, the constitution of the alkaloids in pomegranate bark is accurately known. None of these bases is optically active. Hess was unable to find the active alkaloids described by Tanret and Piccini. It is probable that impurities were responsible for the mistaken conclusion that optically active bases were present. The bases of known constitution occurring in the bark of *Punica granatum* are the following.



¹ K. Hess. Alkaloids of the Pomegranate Tree. I. Pelletierine. *Berichte d. Deutsch. chem. Ges.* 50 (1917), 368; II. Methyl-pelletierine of Tanret and Iso-methyl-pelletierine of Piccini. *Ibid.* 50 (1917), 380; III. Constitution of Pelletierine. *Ibid.* 50 (1917), 1192; IV. Method of Separation for the Purification of Pelletierine Alkaloids. *Ibid.* 50 (1917), 1386; V. Cleavage of Pelletierine and Methyl-isopelletierine into Their Optical Isomers. Explanation of Tanret's Bases. *Ibid.* 51 (1918), 741; VI. Relations of Methyl-isopelletierine, etc. *Ibid.* 52 (1919), 964; VII. Natural Occurrence of isopelletierine. *Ibid.* 52 (1919), 1005.



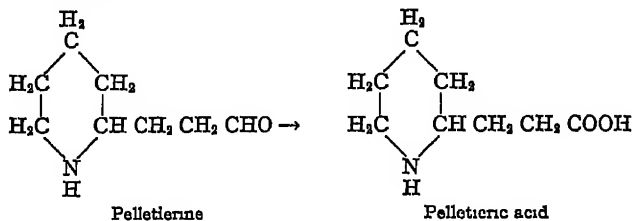
Isolation of Alkaloids by Hess and Eichel (IV Paper)

To get total alkaloids from the bark into solution, add milk of lime to the finely powdered bark and extract with chloroform. Then extract the alkaloids from chloroform solution with dilute sulphuric acid. Add excess of sodium hydroxide solution to this acid aqueous solution of alkaloidal sulphates and extract with ether in a Kempf apparatus. Dry the ether solution with potassium carbonate, filter to remove particles of resinous matter, and fraction *in vacuo*. The first portion of distillate contains lower boiling fractions. The fraction from about 100–120° is a mixture of pelletierine and methyl-isopelletierine. At 145° pseudo-pelletierine begins to distil and solidifies in the receiver to a crystalline mass. Distillation at this point is stopped. The residue in the distilling flask is dissolved in petroleum ether and the solution placed in ice. Pure pseudo-pelletierine crystallizes. To separate pelletierine and methyl-isopelletierine, treat the fraction containing these alkaloids with ethyl-chloro-formate, $\text{Cl CO O C}_2\text{H}_5$. The secondary base, pelletierine, forms a urethane, whereas the tertiary base, methyl-isopelletierine, does not. If the mixture is now fractionally distilled, methyl-isopelletierine boils at 105–106° (15 mm) and pelletierine urethane at 169–170°. The latter heated with fuming hydrochloric acid at 125–135° gives pure pelletierine through elimination of ethyl chloride and carbon dioxide. On the other hand, if crude pelletierine urethane is saponified with alcoholic sodium hydroxide, the liberated base is completely resinified with formation of non-volatile, resinous products. But isopelletierine urethane present in small quantity is readily saponified and this alkaloid can then be distilled.

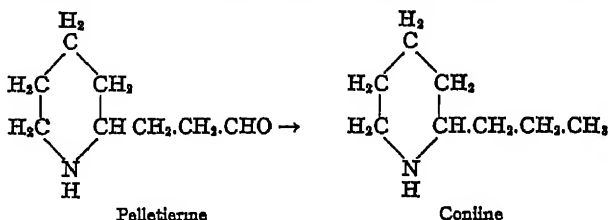
The picrates of the alkaloids in pomegranate bark are especially characteristic, for they are well-crystallized and have definite melting-points. Pelletierine picrate melts at 150°; isopelletierine picrate at 154°, methyl-isopelletierine picrate at 155°; and pseudo-pelletierine picrate at 252–253°.

Pelletierine, $\text{C}_8\text{H}_{15}\text{NO}$, is a colorless oil absorbing oxygen from air with avidity and turning brown from resinification. It has an alkaline reaction and a characteristic spicy aromatic odor. It is soluble in 20 parts of water and in ether, alcohol and chloroform in all proportions. Pelletierine sulphate, $(\text{C}_8\text{H}_{15}\text{NO})_2 \cdot \text{H}_2\text{SO}_4$, a white crystalline non-hygroscopic compound, and pelletierine tannate are used in medicine as a remedy for tapeworm. The nitrogen of pelletierine, according to Hess, is secondary, since it forms an N-acetyl- and an N-benzoyl-derivative that crystallize especially well. Presence of an aldehyde group has also been shown, since pelletierine forms an oxime. Cleavage of water from the latter by treatment with phosphorus pentachloride gives a nitrile that may be

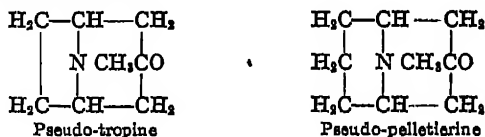
converted by saponification into p-hexahydro-2-pyridyl-propionic acid (pelletieric acid) obtained synthetically



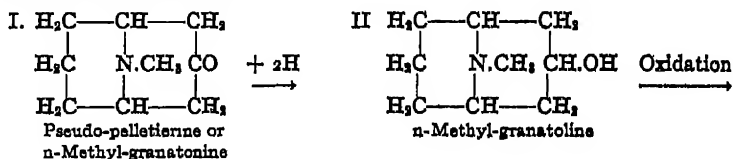
Pelletierine is evidently an oxidation product of coniine. Hess and Eichel in fact succeeded in converting pelletierine into coniine. Pelletierine is converted quantitatively by hydrazine hydrate into a hydrazone. The latter heated with sodium ethylate is converted nearly quantitatively into racemic coniine.

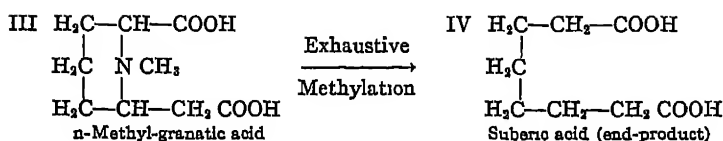


Pseudo-pelletierine, $\text{C}_9\text{H}_{15}\text{NO} + 2\text{H}_2\text{O}$, occurs mainly in the bark of the trunk of *Punica granatum* and in smaller quantity in the root. It forms colorless crystals that melt at 48° and readily dissolve in water, alcohol and ether. As a rule its salts crystallize well. It is both a tertiary base and a ketone, the next higher homologue of pseudo-tropine:



Ciamician and Silber found the constitution of pseudo-pelletierine (I) to be that of a ketone, n-methyl-granatonine. As such it is converted by the action of nascent hydrogen, that is, by sodium amalgam, or sodium and alcohol, into the corresponding secondary alcohol, n-methyl-granatoline (II). The latter is oxidized by chromic acid to n-methyl-granatic acid (III) from which the nitrogen atom may be removed by exhaustive methylation giving as the final product suberic acid (IV).





From 100 kilograms of the bark of *Punica Granatum* C Hess obtained:

50	grams Pelletierine hydrobromide	= 32	grams Pelletierine base	}
32	grams Pelletierine urethane	= 20.5	grams Pelletierine base	
				total 52.5 grams,
179	grams Pseudo-pelletierine,			
22	grams Methyl-isopelletierine,			
about 1.5	grams Isopelletierine,			
about 1	gram 1-(α -N-methyl-piperidyl)-propane-2-one			

So about 0.25 per cent of alkaloids may be obtained in pure condition. The mixture of crude alkaloids amounts to about 0.4 per cent. The alkaloid that preponderates in the plant is pseudo-pelletierine. In view of the fact that a considerable quantity of resinous material remains in the distilling-flask from the distillation of the crude alkaloids, and since most of this product very likely comes from the highly sensitive pelletierine, there is reason to believe that the latter is the chief alkaloid of the pomegranate tree. Isopelletierine and 1-(α -N-methyl-piperidyl)-propane-2-one occur only in insignificant quantities. Hess regards these two alkaloids as intermediate stages in the formation of methyl-isopelletierine and pseudo-pelletierine and consequently they disappear soon after formation.

Therapeutic Use and Toxic Action of Pelletierine

Of the pomegranate alkaloids, pelletierine is the most potent and the one used most frequently as a remedy for tapeworm. For rabbits per kilogram of body-weight the lethal dose is 12 mg. injected subcutaneously. The effects of the poison upon man, and upon warm-blooded animals in general, are muscular rigidity like that caused by veratrine and convulsions. Pelletierine may also produce in man diarrhoea with vomiting, colic, slow pulse, collapse, profuse sweat, headache, muscular rigidity, but particularly disturbances of vision. Even medicinal doses of 0.4–0.5 gram of pelletierine sulphate or tannate given for tapeworm may give rise to slight temporary effects of this nature. Rigor and fever, as quite troublesome secondary effects resulting from use of a decoction of pomegranate bark, have also been repeatedly observed. Pelletierine as well as isopelletierine has a very powerful toxic action upon tapeworm. Usually 0.3–0.4 gram of pelletierine sulphate or tannate is sufficient to effect a cure without producing at the same time very severe toxic effects.

Detection

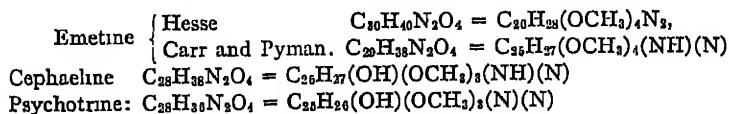
The free alkaloids of pomegranate bark are soluble in ether and chloroform and consequently in the Stas-Otto process they appear in the ether extract of the alkaline aqueous solution. The free bases are volatile with steam. Their odor is characteristic and recalls coniine. Of the general alkaloidal reagents

picric acid would seem to be the best to use as a precipitant, for the picrates of pelletierine and pseudo-pelletierine crystallize well and also have definite melting-points (see above) At the present time the alkaloids of pomegranate bark are without reactions of identification Concentrated sulphuric acid and the reagents of Erdmann, Froehde and Marquis fail to give characteristic colors

Alkaloids of Ipecacuanha

True ipecacuanha root from Urugua ipecacuanha certainly contains three alkaloids and, according to Hesse,¹ five Emetine and cephaeline are present in largest quantity and give rise to the most notable physiological properties of this root In a total alkaloidal content of 2.7 per cent., Carr and Pyman² found in ipecacuanha 1.35 per cent of emetine, 0.25 per cent of cephaeline and a smaller quantity of psychotrine

The composition of these alkaloids is as follows



Carr and Pyman and also Karrer³ assign to emetine the empirical formula, $\text{C}_{20}\text{H}_{40}\text{N}_2\text{O}_4$ Cephaeline differs in composition from emetine in having one less methylene (CH_2) group Emetine has been definitely established as a secondary-tertiary base.

Paul and Cownley have also obtained emetine, cephaeline and psychotrine from ipecacuanha Separation of psychotrine depends upon the fact that unlike emetine and cephaeline it is almost insoluble in ether Consequently when an aqueous extract of the drug is made alkaline and extracted with ether, psychotrine is not removed But it may be extracted with chloroform Moreover cephaeline being a phenol is soluble in sodium hydroxide solution and so cannot be extracted by ether from a solution to which this alkali has been added On the contrary, emetine under these conditions is dissolved by ether If the sodium hydroxide solution is acidified with hydrochloric acid and again made alkaline with ammonia, ether will then extract cephaeline After two or three trials of this method depending upon this difference in behavior toward sodium hydroxide, it is possible to make an approximately quantitative separation of the two alkaloids, since emetine too is somewhat soluble in sodium hydroxide (O Keller) By their method, Paul and Cownley found in Rio root 1.45 per cent of emetine, 0.52 per cent. of cephaeline, and about 0.04 per cent. of psychotrine, in Carthagena root 0.89 per cent. of emetine, 1.25 per cent of cephaeline, and 0.06 per cent of psychotrine

¹ O. Hesse Contribution to the Knowledge of the Alkaloids of True Ipecacuanha. *Annalen der Chemie* 405 (1914), 1

² Carr and Pyman Ipecacuanha Alkaloids. *Journ Chem. Soc.* 105 (1914), 1591

³ P. Karrer Ipecacuanha Alkaloids *Berichte der Deutsch. chem. Ges.* 49 (1916), 2057

Hesse's Isolation of Ipecacuanha Alkaloids.—Mix finely ground ipecacuanha with sufficient cold sodium carbonate solution (1 part of anhydrous sodium carbonate 3 parts of water) so that it is well moistened but does not gather into balls. Then extract at 60–70° with about 8 times its weight of benzene-benzine (1 5). Remove the free alkaloidal bases from the latter solution by means of 0.1 n-sulphuric acid. Add ammonia in slight excess to this solution and take up the free bases in ether. As a rule six extractions are sufficient to remove emetine and cephaeline completely from the root with hot benzene-benzine and also with ether from the alkaloidal sulphate solution rendered alkaline with ammonia. Distil the ether solution to about one-third of its original volume and extract repeatedly with 0.2 n-sodium hydroxide solution to effect the separation of cephaeline from emetine. Cephaeline is extracted completely and only traces of emetine. Continue extraction with 0.2 n-sodium hydroxide solution until the latter tested with ammonium chloride shows no turbidity. Dilute the alkaline cephaeline solution with an equal volume of water, add hydrochloric acid in slight excess, and saturate with ammonia. Free cephaeline is precipitated, taken up in ether, and obtained in long needles by allowing this solution to evaporate.

Mix the ether solution of emetine, from which cephaeline has been removed, with an ether solution of oxalic acid as long as this produces a white precipitate usually flocculent. To observe the effect of this reagent better, add it at intervals of one minute. If the oxalate precipitate is flocculent, addition of a few drops of water will make it more compact. Decant the ether from the oxalate precipitate and dissolve the latter in warm water. If 500 grams of ipecacuanha are taken, 50–60 cc. of warm water will dissolve the oxalate precipitate. Add to this solution saturated aqueous sodium bromide solution until it produces a milky turbidity. Emetine is precipitated as hydrobromide. This dissolves with difficulty in cold water and is filtered off after 48 hours. Addition of ammonia to the solution thus obtained produces a white, flocculent precipitate consisting in reality of the alkaloid called ipecamine by Hesse. To remove attendant impurities from precipitated emetine hydrobromide, crystallize it several times from hot water adding at first a little sodium bromide. Finally the hot solution of hydrobromide is cooled and treated with a slight excess of ammonia. Emetine appears as a white flocculent precipitate that soon gathers into a dense mass. Protect this precipitate well from light, otherwise it will turn yellowish or even yellow. Emetine well washed with cold water and air-dried retains about 1 per cent. of moisture easily removed in desiccator over sulphuric acid.

Emetine

Emetine is precipitated from solutions of its salts by ammonia and potassium or sodium hydroxide solution in white flocks that form a white powder when air-dried, or a white mass that is easily reduced to powder. As yet emetine has not been obtained in crystalline form. Emetine is nearly insoluble in cold water, dissolves but slightly in water containing ammonia, and potassium or sodium hydroxide, and is easily extracted from these solutions by ether or chloroform. It is readily soluble in alcohol, ether, acetone, chloroform, and hot benzene. Emetine solutions exposed to sunlight quickly turn yellow. Solutions in dilute acids are also gradually colored by light. An alcohol solution of emetine is

laevo-rotatory Emetine salts have the following characteristics Some of these compounds crystallize well, 1 molecule of alkaloid combining with 2 molecules of monobasic acid or with 1 molecule of dibasic acid Emetine therefore is a di-acid base Emetine hydrobromide, $C_{28}H_{40}N_2O_8 \cdot 2HBr \cdot 4H_2O$, crystallizes in white needles that dissolve very easily in hot water but only slightly in cold water and alcohol In desiccator it completely loses its water of crystallization. It is dextro-rotatory

Detection of Emetine.—Concentrated sulphuric acid dissolves pure emetine without color. If, however, sulphuric acid containing molybdic acid, that is, Froehde's reagent, is added to this solution, it develops an intense emerald-green color within a few minutes. This color persists for days, if the solution is kept in desiccator protected from moist air.

Cephaeline

Cephaeline crystallizes from ether in long, delicate, white needles that easily dissolve in ether, alcohol, acetone, chloroform, methyl alcohol, and hot benzene when freshly precipitated Mixed with 0.5 n-potassium hydroxide solution in equal molecular proportions, cephaeline only partly dissolves, since the potassium salt is formed as the crystals in part swell up. Gentle warming at once causes solution from which ether will not extract cephaeline But ether will dissolve all the cephaeline following addition of an equivalent quantity of ammonium chloride Added to solutions of the alkaloid in dilute acids, potassium or sodium hydroxide solutions produce white, flocculent precipitates that redissolve in excess of precipitant This behavior establishes cephaeline as a phenolic base. Cephaeline has a faintly bitter taste and in alcoholic solution reacts strongly alkaline toward litmus Its alcoholic solution is laevo-rotatory. $[\alpha]_D^{15} = -21.2^\circ$ Cephaeline completely neutralizes acids. One molecule of alkaloid combines with 2 molecules of monobasic acid or with 1 molecule of dibasic acid

Detection of Cephaeline.—A little ferric chloride solution added to an alcoholic cephaeline solution produces a red-brown color that soon changes to blue-green as cephaeline hydrochloride forms. Concentrated sulphuric acid dissolves pure cephaeline without color. Addition of a solution of pure ammonium molybdate in concentrated sulphuric acid (sp. gr. 1.84) at once colors the cephaeline solution brown-red soon changing to blue, finally to green, and fading out in a few hours.

Calcium Hypochlorite Test.—Calcium hypochlorite solution develops color when added to solutions of ipecacuanha alkaloids in dilute hydrochloric acid, that given by emetine being yellow and by cephaeline red-yellow. In this test Keller uses glacial acetic acid as the solvent of the two alkaloids.

Psychotrine

When the ipecacuanha alkaloids are extracted by amyl alcohol and removed from this solvent with dilute sulphuric acid, psychotrine, according to Paul and Cownley,¹ remains in this acid solution after it has been saturated with ammonia and shaken with ether which extracts emetine and cephaeline. This is also the case, as mentioned above, when benzene-benzine mixture is used in Hesse's method in place of amyl alcohol. Traces of psychotrine of course pass into the ether. The aqueous ammonia solution is extracted with small quantities of chloroform as long as it continues to show blue fluorescence. The chloroform solution is then shaken with dilute sulphuric acid and the latter solution saturated with a little ammonia. This produces a yellowish, amorphous precipitate that soon changes into small crystals. Finally, psychotrine is recrystallized from a little hot alcohol or acetone mixed with four times the volume of water. Psychotrine, $C_{28}H_{36}N_2O_4 = C_{28}H_{36}(OCH_3)_2(OH)(N)(N)$, is obtained in splendid crystals having the lustre of diamonds. But they effloresce losing 4 molecules of crystal water and at the same time their lustre. Viewed in one direction these crystals appear colorless or yellowish, in other directions red or steel-blue. Anhydrous psychotrine dried at 100° melts at 122° . Crystalline psychotrine is practically insoluble in ether but when first precipitated freely dissolves in this solvent soon separating again except for traces. It dissolves freely in alcohol, acetone and chloroform. Upon addition of water, psychotrine separates from alcohol or acetone in splendid crystals, $[\alpha]_D^{15} = +69.3^\circ$. The ammonia solution of psychotrine, like solutions in water, alcohol or acetone, has a strong blue fluorescence. Psychotrine is precipitated from solutions of its salts by sodium or potassium hydroxide solution in white flocks easily soluble with heat in excess of alkali. It has a very bitter taste and easily causes nausea. In alcoholic solution psychotrine completely neutralizes hydrochloric and hydrobromic acids. A little ferric chloride added to an aqueous solution of the hydrochloride produces a blue color. In this respect it behaves like the phenolic base morphine. Upon reduction in hot alcohol with sodium, psychotrine takes up 2 atoms of hydrogen giving cephaeline and isocephaeline (Carr and Pyman).

Reactions and Detection of Emetine and Cephaeline²

Concentrated nitric acid dissolves emetine and cephaeline with orange-yellow color.

Glacial acetic acid in presence of a little calcium hypochlorite solution dissolves emetine yellow and cephaeline red-yellow.

Concentrated sulphuric acid with addition of a few drops of ferric chloride solution dissolves emetine and cephaeline yellow with a tinge of green.

¹ R. H. Paul and A. J. Cownley: *Chemistry of Ipecacuanha*. Pharmac. Journ. and Transact. (3) 25 (1894/95,) 111 and 690.

² A. H. Allen and G. E. Scott-Smith: *Certain Reactions of the Alkaloids of Ipecacuanha*. Pharmaz. Journ. (4) 15 (1902), 552.

Formaldehyde-sulphuric acid dissolves emetine with green color that turns yellow after 4 hours, cephaeline is deep yellow at once and the color is stable.

Froehde's reagent dissolves emetine with a splendid emerald-green color that persists for more than 24 hours. Cephaeline gives deep red-violet becoming brown, olive-green, green, and later yellow. The same reagent containing 1 drop of fuming hydrochloric acid dissolves emetine with green color having a faint yellow tint, cephaeline intense blue-green. Emetine allowed to stand with Froehde's reagent in a shallow dish gives a splendid emerald-green slowly turning dark blue from the margin, cephaeline violet quickly turning dark blue from the margin.

The behavior of these alkaloids with Froehde's reagent, according to Allen-Scott-Smith, is especially characteristic when carried out in the following manner: emetine gives a dirty green color changing to light grass-green on addition of hydrochloric acid, cephaeline a purple color changed by hydrochloric acid to Prussian blue; and psychotrine a dark purple changed by hydrochloric acid to pale green. The mixed alkaloids of *ipecacuanha* also give with great distinctness the Prussian blue reaction of cephaeline upon addition of hydrochloric acid and in this way they may be distinguished from the opium alkaloids.¹

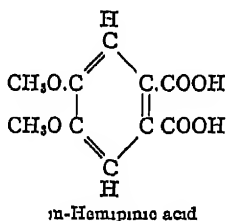
Reduction Test.—The alkaloids of *ipecacuanha* as derivatives of catechol exert a reducing action, for example, upon a mixture of potassium ferricyanide and ferric chloride with formation of a blue color. A precipitate of Prussian blue is obtained when cephaeline or psychotrine is used. Obviously this test is not characteristic of the alkaloids of *ipecacuanha*, for morphine behaves in the same way.

Constitution of Emetine and Cephaeline

Methoxyl-groups in emetine and cephaeline are easily determined by Zeisel's method. This test shows that emetine contains four and cephaeline only three such groups. Karrer states that these demethylated compounds may be easily isolated and he has shown that the new base thus obtained from cephaeline is identical in every respect with the base obtained in the same way from emetine and called emetoline. With a drop of ferric chloride solution the liberated compound purified from alcohol gives a deep green color changing to violet upon addition of ammonia. It also reduces ammoniacal silver nitrate solution even in the cold. In other words, it manifests all the catechol reactions. Since emetine has four and cephaeline three methoxyl-groups, emetine is simply

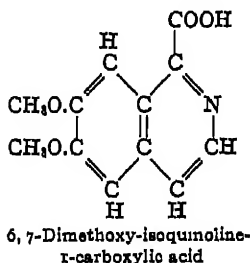
¹ O. Keller: Researches upon the Alkaloids of *Ipecacuanha*. *Archiv d Pharmazie* 149 (1911), 51.

the methyl ether of cephaeline, that is, in cephaeline three of the four hydroxyl-groups are methylated, whereas in emetine all four are methylated! Emetine therefore bears the same relation to cephaeline that codeine does to morphine. A phenolic hydroxyl-group is present in the cephaeline molecule. This much may be said with regard to the relative positions of these four groups, that at least two are ortho to each other, for emetine gives the reactions of catechol. This view is also in accord with the formation of *m*-hemipinic acid by oxidizing emetine (Windaus)



In accordance with the above results methylation of cephaeline should give emetine. Karrer has shown that this is the case. Gentle warming in a tube for several hours of a methyl alcohol solution of cephaeline, containing 2 atoms of sodium with 1 molecule of dimethyl sulphate, gives a good yield of an alkali-insoluble compound. Pure emetine may be obtained from this product by means of the beautifully crystalline hydriodide. Emetine prepared in this way is entirely identical in chemical and biological properties with emetine as it occurs in nature. Cephaeline may be methylated better and more simply by means of nascent diazo-methane.

On the basis of the researches of Koller, Hesse, Carr and Pyman, the nitrogen atoms in emetine and also in cephaeline have been shown in one case to be tertiary and in the other secondary. Most likely one nitrogen atom at least is in an isoquinoline ring, for Carr and Pyman found among the oxidation products of emetine a very small quantity to be sure of 6,7-dimethoxy-isoquinoline-1-carboxylic acid. One of the two nitrogen atoms of emetine belongs to two rings at the same time (Karrer).



Physiological Action and Therapeutic Use

Emetine is an excellent remedy that is specific for amoebic dysentery. Emetine hydrochloride or hydrobromide has proved an excellent expectorant in the

treatment of bronchitis and other catarrhal affections of the respiratory tract. Introduced into the stomach in rather large doses of 0.015 gram, the hydrochloride to be sure causes emesis after about three-quarters of an hour, but not in doses of only 0.005 gram. Cephaline hydrochloride causes severe vomiting even in doses of only 0.005 gram. According to Lewin, cephaline is the best emetic, emetine a powerful expectorant, whereas psychotrine is said to have no specific action. The use of emetine salts as a remedy for amoebic dysentery is of very great importance, for this malady is promptly cured by subcutaneous injection of emetine hydrochloride, or hydrobromide, without irritation of the mucous membranes at the same time (Rogers). The total daily dose is 0.03-0.04 gram. Emetine is effective because it kills *Entamoeba dysenterica*, the cause of this dysentery, and thus renders it innocuous. On the contrary, emetine is without effect upon *Bacillus dysentericus*, the cause of bacilli-dysentery. Subcutaneous injection of emetine salts speedily cures chronic dysentery arising from amoebic dysentery. Emetine, according to Pick and Wasicky,¹ causes paralysis of the smooth muscles of the blood-vessels of the frog and mammals, also of the muscles of the digestive tract, the bronchial muscles, and finally the protozoa. This paralyzing action does not make the nerves but only the muscles the point of attack. Paralysis caused by emetine is counteracted by barium chloride and vice versa the effect of barium is removed by emetine.

YOHIMBINE

Yohimbine, $C_{21}H_{21}N_3O_4$, together with other alkaloids occurs in Yohimbe bark obtained from West Africa. This alkaloid was first obtained in a state of purity by L. Spiegel in 1896. It appears in white needles melting at 234° and turning yellowish upon exposure to light. It is almost insoluble in water but freely soluble in alcohol, ether and chloroform. Yohimbine at 120° forms the anhydride, $C_{21}H_{20}N_3O_3$. The hydrochloride of this latter body, $C_{21}H_{20}N_3O_3 \cdot HCl$, used in medicine, is formed when the base is converted into its hydrochloride. Yohimbine is a dextro-rotatory, tertiary base containing one methoxyl and one hydroxyl-group. If the alkaloid is allowed to stand for 24 hours in contact with potassium hydroxide solution, it loses methyl alcohol and is converted into the potassium salt of yohimbic acid. On the other hand, a mixture of free yohimbic acid and methyl alcohol saturated with dry hydrochloric acid gas yields a precipitate of yohimbine hydrochloride. Upon the basis of this behavior yohimbine is to be regarded as the methyl ester of yohimbic acid.

Physiologically yohimbine bears a certain resemblance to cocaine, for like the latter alkaloid it acts as a local anaesthetic and additionally causes enlargement of the blood-vessels. It enlarges the blood-vessels of the skin, kidneys and intestines and increases the flow of blood to the external genitals. After internal administration, Fr. Müller observed an action upon respiration, the circulation of the blood, and the genital reflexes. Even in small doses it causes increased irritability of the respiratory centre.

If the general procedure for detecting organic poisons by the Stas-Otto process is followed, yohimbine will be extracted by ether or chloroform from the aqueous solution made alkaline with sodium hydroxide solution.

¹ E. Pick and R. Wasicky. *Archiv f. experim. Pathol. und Pharmacol.* 80 (1916), 147.

Detection

Yohimbine reduces ammoniacal silver nitrate solution. As a rule the precipitates given by this base with general alkaloidal reagents are heavy. Concentrated sulphuric acid dissolves yohimbine without color. A crystal of potassium dichromate introduced into this solution produces violet-red streaks. With the reagents of Froehde, Mecke and Mandelin a deep blue color appears at once. Beginning at the margin this color gradually changes to green. A drop of Meller's reagent (1 gram of benzaldehyde dissolved in 4 grams of absolute alcohol), added to yohimbine on a watch-glass and followed by 1-2 drops of concentrated sulphuric acid, produces at first a dark brown color. Beginning at the margin this color gradually changes to cherry-red and violet, the entire liquid finally becoming violet.

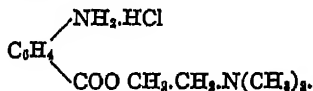
COCAINE SUBSTITUTES

The compounds to be considered in this connection are: Novocaine, Orthoform, Stovaine, Tutocaine, Psicaine, Tropacocaine, together with Adrenaline.

Within recent years various artificial cocaine substitutes have been recommended as local anaesthetics. Belonging to this class are such compounds as acoine, alpine, anaesthesine, eucaine B, holocaine, nirvanine, novocaine, orthoform, stovaine, tutocaine, psicaine, and many others. All these synthetic cocaine substitutes like cocaine itself are bases that can be extracted by ether or chloroform from an alkaline aqueous solution. Consequently if the Stas-Otto process is followed, these bases will be found in the ether extract where the true alkaloids appear. Most of the general alkaloidal reagents produce precipitates in solutions of their salts. Like cocaine the free bases as well as their salts cause temporary insensibility when applied to the tongue.

NOVOCAINE

Novocaine is the hydrochloride of the dimethyl-amino-ethyl ester of para-amino-benzoic acid:



This compound crystallizes in colorless, odorless needles that have a faintly bitter taste. It dissolves in 1 part of water, 30 parts of alcohol, and with great difficulty in ether. Its aqueous solution has a neutral reaction. Alkalies, alkaline carbonates, but not alkaline bicarbonates, precipitate the free base from novocaine solutions as an oil that quickly becomes crystalline. This oil is insoluble

ble in water but readily soluble in alcohol, ether, benzene, and chloroform. The free base melts at $61-63^{\circ}$, novocaine at 155° . The picrate crystallizes in yellow needles melting at 156° . Ether easily extracts the free base from a solution rendered alkaline with sodium hydroxide solution.

Reactions

1. Diazonium Reaction.—Novocaine is a primary aromatic base and can be diazotized. The diazonium compound may then be coupled with phenols, thus forming azo-dyestuffs. Acidify an aqueous novocaine solution with hydrochloric acid. Then add a few drops of 0.5 per cent. sodium nitrite solution and finally β -naphthol dissolved in excess of sodium hydroxide solution. This produces a scarlet-red color. Cocaine, acoine, alipine, eucaine, holocaine, stovaine and psicaine do not give this reaction. The primary amine anaesthesia gives a strong and nirvanine a very weak color reaction. Detection of novocaine in presence of cocaine is based upon this diazonium reaction (see page 191).

2. Calomel Test.—Addition of a few drops of diluted alcohol to an intimate mixture of equal quantities of novocaine and calomel after some time causes a blackening to appear. Cocaine behaves in a similar manner.

3. Iodoform Test.—Cleavage of the ethyl group from novocaine takes place easily and consequently the odor of iodoform is apparent when the precipitate produced by iodo-potassium iodide is dissolved in sodium hydroxide solution and then gently warmed.

The following reactions serve to differentiate novocaine from cocaine (Gadamer):

(a) Add a few drops of 5 per cent. mercuric chloride solution to 0.1 gram of substance dissolved in 5 cc. of water. Novocaine gives a white precipitate soluble in a little dilute hydrochloric acid. Cocaine gives a white precipitate insoluble in a few drops of hydrochloric acid.

(b) Add a drop of 1 per cent. potassium permanganate solution to 0.25 gram of substance dissolved in 5 cc. of water. At first the novocaine solution remains clear and for a short time violet. Then with loss of color a separation of $MnO_2 \cdot H_2O$ takes place. Cocaine gives a violet precipitate of cocaine permanganate. Reduction takes place only after very long standing.

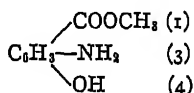
(c) Add 5 drops of 3 per cent. chromic acid solution to 0.05 gram of substance dissolved in 5 cc. of water. The color of the novocaine solution at first does not fade; later turbidity appears and fading

that is arrested by addition of hydrochloric acid. The precipitate in the cocaine solution at first continues to disappear and upon acidification remains as yellow, resinous cocaine chromate.

Physiological Action.—An aqueous novocaine solution (1 + 1) dropped upon mucous membranes, or injected subcutaneously, produces temporary but intense anaesthesia. Pupils, accommodation and blood vessels are unaffected. At the highest, the toxicity of novocaine is one-sixth as much as that of cocaine. Its action upon the nerve-roots is the same as that of cocaine.

ORTHOFORM (New)¹

Orthoform is the methyl ester of meta-amino-para-oxy-benzoic acid:



This compound is a white or yellowish crystalline powder without odor or taste. Applied to the tongue it does not produce insensibility. Almost insoluble in water, it dissolves easily in 6 parts of alcohol, 50 parts of ether, as well as in benzene and chloroform. Orthoform at the same time a base and a phenol is dissolved both by acids and alkaline hydroxides. In aqueous solution its salts with acids have an acid reaction, an acidulous taste, and a weak anaesthetic action upon the tongue. Solutions in alkaline hydroxides turn red in the air from oxidation. The commercial product melts at 142°. Because of the feeble basic nature of orthoform its salts with acids are hydrolyzed to such an extent in aqueous solution that in the Stas-Otto process a very considerable quantity of the base can be extracted from a tartaric acid solution. Ether will not remove orthoform from a solution made alkaline with sodium hydroxide but extracts it easily from a solution rendered alkaline with sodium bicarbonate.

Reactions

1. **Diazonium Reaction.**—Orthoform is a primary aromatic amine and can be diazotized. A few drops of sodium nitrite solution turn a solution of orthoform in dilute hydrochloric acid greenish yellow. Addition of a strongly alkaline solution of β -naphthol produces a fine red color due to formation of an azo-dyestuff.

2. **Ferric Chloride Test.**—The violet color imparted by a little ferric chloride to an alcohol solution of orthoform becomes brown with more of the reagent and the aqueous filtered extract of it green.

3. **Nitric Acid Test.**—Addition of a drop of nitric acid to orthoform rubbed with a little concentrated sulphuric acid colors the mixture red-violet or blue-violet. Excess of sodium hydroxide solution changes this color to red.

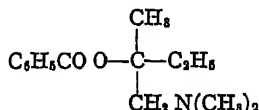
¹ "Orthoform Old" is isomeric with "Orthoform New."

4. **Lead Peroxide Test.**—Lead peroxide colors a glacial acetic acid solution of orthoform green.

Physiological Action—In general, esters of aromatic oxy-amino-benzoic acids, such as orthoform, possess the property of causing local anaesthesia. On account of its insolubility, orthoform cannot be used subcutaneously. According to Kobert, the two orthoforms occasionally give rise to extensive necroses. They produce no anaesthesia upon unbroken and mucous surfaces of the body. The two orthoforms find no application in work upon the eye.

STOVAINE

Stovaine is the hydrochloride of the dimethylamino-ethyl-isopropyl ester of benzoic acid



Stovaine resembles cocaine in having two atomic complexes that are the same, that is, a methylated tertiary amino-group and the benzoic acid ester of an alcohol. It is a white crystalline powder. Because of decomposition the faint odor of an amine base is apparent. Stovaine has a bitter taste and applied to the tongue causes temporary anaesthesia. It is very easily soluble in water and dissolves in 5 parts of methyl alcohol, 70 parts of absolute alcohol, and 50 parts of chloroform. In ether and acetone it is almost insoluble. It melts at 175–176°. The free base is a colorless oil dissolving very freely in alcohol and ether and almost insoluble in water. Ether extracts it from aqueous stovaine solutions that have been rendered alkaline with sodium hydroxide solution. The picrate of stovaine crystallizes well.

Reactions

General alkaloidal reagents give precipitates with aqueous stovaine solutions. Detection of stovaine depends mainly upon recognizing the presence of the benzoyl-group. Heat the material in a boiling water-bath for a few minutes with a few drops of alcohol and concentrated sulphuric acid. If stovaine is present, the odor of ethyl benzoate will be detected, especially if a little water is cautiously added to the mixture. The following reactions serve to differentiate stovaine from cocaine:

(a) Saturated aqueous picric acid solution gives with cocaine fine crystalline needles melting at 165–166°. Stovaine forms tabular crystals melting at 115–116°.

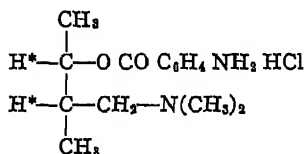
(b) Add 5 drops of 1 per cent. potassium permanganate solution to 0.1 gram of substance dissolved in 5 cc. of water. The violet

crystalline precipitate given by cocaine even after 0.75 hour retain its color unchanged. With stovaine the solution remains clear and after 0.75 hour the color disappears entirely with separation $\text{MnO}_2 \cdot \text{H}_2\text{O}$.

Physiological Action.—Applied to mucous membranes stovaine produces intense local anaesthesia. It does not cause contraction of the blood-vessels. Following medicinal doses of 0.02–0.05 gram, the heart-beat is slowed and strengthened rather than weakened. According to Kobert, the toxic effects consist of tonic and clonic convulsions that may terminate in collapse. The lethal dose is said to be twice as great as that of cocaine. Introduction of 1 per cent stovaine solutions into the eye produces not only anaesthesia but a slight enlargement of the pupil and accommodation disturbances. In the case of the rabbit, 4 per cent stovaine solution causes removal of the epithelium of the cornea (de Lapersonne). Following injection of 2 per cent stovaine solution Sinclair observed in 4 patients appearance of local gangrene.

TUTOCAINE

Tutocaine¹ is the hydrochloride of p-amino-benzoyl- α -dimethylamino-methyl- γ -butanol, that is, it is a derivative of an α -amino- γ -alcohol and has following structure



The presence of two asymmetric carbon atoms (*) makes possible several isomeric forms of tutocaine. The commercial product is a racemic compound, the hydrochloride of which melts at 213–215° and forms fine, white, microscopic needles that are soluble in water at 20° to the extent of 15.5 per cent. Its aqueous solution is colorless and neutral and during sterilization does not undergo decomposition. Tutocaine dissolves with difficulty in alcohol.

Reactions

1. Sodium hydroxide solution, added to an aqueous tutocaine solution warms to about 50°, precipitates a yellowish oil.

2. Mercuric chloride produces a white and iodine solution a brown precipitate. Silver nitrate precipitates white silver chloride.

3. **Azo-dye Test.**—As a primary aromatic amine, tutocaine can be diazotized and converted into an azo-dye. Dissolve 0.1 gram of tutocaine in 5 cc. of water, add 2 drops of hydrochloric acid, and then 2 drops of sodium nitrite solution. Add this mixture to a solution of 0.2 gram of β -naphthol in 1 cc. of sodium hydroxide solution and 9 cc. of water. A scarlet-red precipitate is produced.

¹ W. Schulemann: Tutocaine as a Surface and Infiltration Anaesthetic. *Klinische Wochenschrift* III, No. 16.

4. **Permanganate-Sulphuric Acid Test**—Add 3 drops of dilute sulphuric acid to a solution of 0.1 gram of tutocaine in 5 cc of water and then 1 drop of dilute potassium permanganate solution. The violet color of the latter should at once disappear.

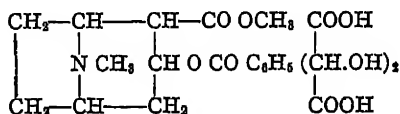
5 If 0.1 gram of tutocaine is dissolved in 1 cc of nitric acid (25 per cent. HNO_3), the solution should have no color.

6 Hydrogen sulphide water added to a solution of tutocaine in water should produce no change.

Physiological Action—In a rabbit's or man's eye, a tutocaine solution causes no irritation whatever. A 2 per cent solution moderately enlarges the blood-vessels of the conjunctiva of the human eye. Slight enlargement of the pupil of the human eye appeared some time after the solution had been introduced. A 1-0.25 per cent solution of tutocaine introduced into the conjunctival sac of a rabbit's eye produced complete anaesthesia of the cornea in about 4 minutes. The action of tutocaine is very persistent. Where a 1 per cent. tutocaine solution was injected in 2 minutes into the auricular vein of the rabbit, the lethal dose was 25-30 mg. Following entrance of tutocaine into the circulation, toxic effects decrease as elimination of the poison takes place which proceeds rapidly in the human organism. In toxicity tutocaine is very close to novocaine. Unlike cocaine it exhibits no tendency toward cumulative action.

PSICAINE

Psicaine¹ is the acid tartrate of dextro-rotatory pseudo-cocaine, or dextro- ψ -cocaine, having the composition $\text{C}_{17}\text{H}_{21}\text{O}_4\text{N}$ $\text{C}_6\text{H}_5\text{O}_6$ and the structural formula.



Psicaine is a white crystalline powder soluble in 4 parts of water and with somewhat more difficulty in alcohol. Aqueous psicaine solutions are acid to litmus, have a bitter taste, and applied to the tongue produce prolonged insensibility. They remain unchanged when sterilized for one hour in a current of steam. Psicaine may be differentiated from ordinary cocaine by its behavior toward polarized light, $[\alpha]_D^{20} = +43^\circ$. In the course of his cocaine synthesis, Willstätter² examined dextro-pseudo-cocaine.

Pharmacological Properties and Uses in Medicine.—Anaesthetic strength is influenced by spatial arrangement of atoms in the cocaine molecule and is increased in the pseudo-series. The activity of psicaine, when brought into contact with peripheral nerve endings, is about twice that of ordinary cocaine. The organism moreover finds itself more easily of psicaine than of cocaine. Animals tolerate twice as much psicaine subcutaneously injected as cocaine without intoxi-

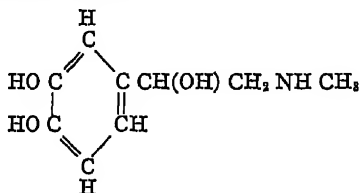
¹ R. Gottlieb. *Archiv f. experim. Path. u. Pharmacol.* 97 (1923), 113; *Münchener med. Wochenschr.* 26 (1924), 850.

² R. Willstätter. *Münchener med. Wochenschr.* 26 (1924), 849; R. Willstätter, O. Wolfes and H. Maeder. *Annalen d. Chem.* 434 (1923), 111.

cation Recovery is more rapid from psicaine than from cocaine intoxication. Except for traces eliminated in the urine, psicaine is entirely destroyed in metabolism, whereas cats eliminate 5-20 per cent of cocaine. Otherwise the pharmacological behavior of psicaine is quite like that of cocaine. Like the latter penetrates well. At first there is enlargement of the blood vessels but addition of adrenaline promptly produces contraction. Psicaine behaves well in conjunction with adrenaline. In small doses cocaine exhibits euphoric action whereas psicaine does not. Consequently appearance of cocaineism is excluded when psicaine is used. Psicaine is used principally as a superficial anaesthetic especially upon mucous surfaces.

SUPRARENINE OR ADRENALINE

Suprarenine, adrenaline, or epinephrine, the active substance of the suprarenal gland, causing contraction of blood-vessels and increase of blood-pressure, is ortho-dioxy-phenyl-ethanol-methyl-ammonium having the following structure:

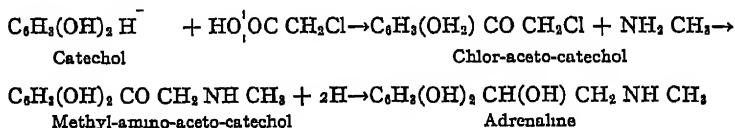


The laevo-rotatory form of adrenaline occurs in the suprarenal gland. This alone finds use in medicine. Its physiological action is manifested in pronounced contraction of blood-vessels, great increase of blood-pressure, slowing of heart action, enlargement of pupils, and increased excretion of urine. Glycosuria may also appear. In addition to changes in pulse, blood-pressure and pupils, Kobert cites a case observed by Ambey in which, after intravenous injection of adrenaline, appeared vomiting, excitement, weak and bloody diarrhoea and death during the picture of prostration.

Laevo-adrenaline is a white crystalline powder easily turns gray in air, or small white needles that decompose at 212° . It has a faintly bitter taste and produces insensibility when applied to tongue. It is difficultly soluble in water with alkaline reaction, less soluble in alcohol, and practically insoluble in ether, benzene and chloroform. At the same time a diatomic phenol and a diamine base, it dissolves both in alkaline hydroxides and acids $[\alpha]_{19.5}^D - 51.4^{\circ}$.

Synthetic Adrenaline.—Catechol gently heated with monochloroacetic acid in presence of phosphorus oxychloride is converted into chlor-aceto-catechol.

latter, kept cold and treated with methyl-amine, is converted into methyl-amino-aceto-catechol. This compound is a ketone and can be reduced to the secondary alcohol, adrenaline.



Synthetic adrenaline obtained in this manner is inactive. It is a white crystalline powder that decomposes at about 230° . By fractional crystallization of the acid dextro-tartaric acid salt, Flächer¹ succeeded in resolving synthetic dl-suprarenine into its two components. In physical, chemical and physiological properties synthetic l-suprarenine is identical with the product isolated from suprarenal glands. The effect of l-suprarenine upon blood-pressure is much greater than that of d-suprarenine. E. Abderhalden and Fr. Müller² have shown that l-suprarenine is about 15 times more active than d-suprarenine. Corresponding to its composition, dl-suprarenine occupies a position midway between the two.

Detection

Suprarenine as a derivative of catechol possesses in general the chemical properties of this compound. It is easily oxidized and consequently is a powerful reducing agent. Suprarenine gives the well-known reduction tests with ammoniacal silver nitrate, gold chloride solution, and with Nessler's reagent. Iodic acid is reduced. Therefore a mixture of suprarenine and iodic acid colors chloroform violet. A mixture of ferric chloride and potassium ferricyanide turns blue from formation of Prussian blue. Phospho-molybdic acid gives a faint green color changing to deep blue with excess of sodium hydroxide solution. This blue color appears especially well if the solution is acidified with hydrochloric acid. Even very dilute adrenaline solutions are colored deep blue by phospho-tungstic acid with addition of excess of saturated sodium carbonate solution. This test is also a reaction of reduction, for under like conditions various reducing substances, such as uric acid, also give blue solutions with phospho-tungstic acid.

Ferric Chloride Test.—Very dilute ferric chloride solution imparts an emerald-green color to a solution of adrenaline, as it does to a catechol solution. Ammonia changes this color to blood-red.

¹ F. Flächer. Cleavage of Synthetic dl-Suprarenine into Its Optically-active Components. *Zeitschr. f. physiol. Chem.* 58 (1908), 189.

² E. Abderhalden and Fr. Müller. Behavior of Blood Pressure after Intravenous Injection of l-, d- and dl-Suprarenin. *Zeitschr. f. physiol. Chem.* 58 (1908), 185.

Concentrated sulphuric acid dissolves adrenaline with yellowish color changed to yellow-red by a drop of 25 per cent. nitric acid. Marquis' reagent dissolves it first with rose-red color that changes later to cherry-red. Froehde's reagent dissolves it first brown and then greenish. Millon's reagent dissolves it with yellow-red color (Kraus¹).

Estimation of Adrenaline According to Folin² in the Suprarenal Gland with the Autenrieth-Königsberger Wedge-Colorimeter³

Weigh finely comminuted glands freed from fat and triturate well in a mortar with fine sand and 0.1 N-hydrochloric acid. Then transfer this mixture without loss to an Erlenmeyer flask, rinsing out the mortar with 0.1 N-hydrochloric acid and water. For every 2 grams of fresh suprarenal glands use a total of 15 cc. of acid and about three times as much water. Heat the mixture to boiling. No coagulation of albumin takes place in presence of so much hydrochloric acid but adrenaline is dissolved. Add to the boiling liquid 5 cc. of 10 per cent sodium acetate solution for every 15 cc. of 0.1 N-hydrochloric acid and continue boiling. This will produce a precipitate containing albumin. Pour the entire mixture, with exception of the sand, into a graduated flask holding 100 cc. for 2 grams of gland, 200 cc. for 2-4 grams, and 500 cc. for 8-10 grams. Cool and fill to the mark with water, rinsing out the Erlenmeyer flask with water. Shake well and pour through a dry filter. Estimate the adrenaline content and for the colorimetric determination put 5-10 cc. of the clear filtrate into a 50 cc. graduated flask, adding 1 cc. of phospho-tungstic reagent and 10 cc. of saturated sodium carbonate solution. Allow the mixture to stand for 10 minutes, fill to the mark with water and shake. By means of a calibrated comparison-wedge determine in the colorimeter mentioned above the tinctorial strength of the blue solution obtained. Fill the comparison-wedge with an artificial color solution that keeps and is optically identical with the blue adrenaline solution to be examined. In this case, for example, a solution of copper tetramine sulphate may be used.

Calibration of the Comparison-Wedge

Calibrate this wedge in the following manner with "Suprareninum crystallissimum purissimum"⁴ supplied in small tubes holding 0.05 gram. Dissolve the contents of such a tube in a little dilute hydrochloric acid and then dilute this solution to 500 cc. with water. 1 cc. = 0.1 mg. of adrenaline. Put 1, 2, 3, etc., cc. of this solution in a 50 cc. graduated flask, adding in each case 1 cc. of

¹ L. Kraus: Certain Color Reactions of Synthetic Suprarenine. *Apoth.-Ztg.* 23 (1908), 701.

² O. Folin, W. B. Cannon and W. Dennis: Colorimetric Method of Estimating Suprarenine. *The Journ. of Biol. Chem.* 13 (1912/13), 477.

³ W. Autenrieth and H. Quantmeyer: Estimation of Adrenaline in the Suprarenal Capsule and of Uric Acid in Blood. *Münch. med. Wochenschrift* 1921, No. 32, 1007.

⁴ A preparation of the Höchst Color Works, formerly Meister, Lucius and Brünig.

phospho-tungstic acid reagent and 10 cc. of saturated sodium carbonate solution. Allow the mixture to stand for 10 minutes, fill to the mark with water and shake. Determine in the colorimeter for each color solution the point at which it is identical with the comparison-wedge in color strength. The following values were determined in this manner:

Adrenaline solution in cc	10	15	20	25	30
Adrenaline in mg	0.1	0.15	0.2	0.25	0.3
Reading for equal color strength	74	64	53	43	33

In each case, record adrenaline in mg upon the abscissae and readings for equal color strength upon the ordinates of a coordinate system. The line connecting points of intersection of coordinates is the calibration curve of the comparison-wedge. With this comparison-wedge, if in each case the color solution is diluted to 50 cc., it is possible to determine quantities of adrenaline from 0.05 to 0.45 mg.

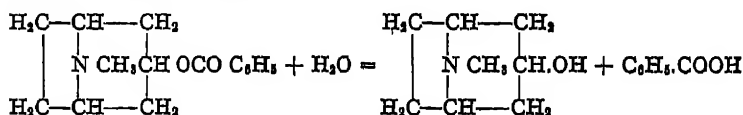
Example.—Weight of the two lobes of the suprarenal glands of the ox = 12.3 grams. Extract solution = 600 cc. Blue color solution prepared from 5 cc of this solution = 50 cc. Equal color strength at 51 on the scale = 0.26 mg of adrenaline. So the adrenaline content of the extract and of the entire suprarenal gland: $120 \times 0.26 = 31.2$ mg

Preparation of Phospho-tungstic Acid Reagent

Heat a mixture of 100 grams of sodium tungstate with 80 cc. of 85 per cent. phosphoric acid and 750 cc. of water to boiling under a reflux. Boil for 4 hours, cool and make up to a liter with water. This reagent should be kept in small dark bottles.

TROPACOCAINE

Tropacocaine, $C_{15}H_{19}NO_2$, is benzoyl-pseudo-tropeine occurring in Java coca leaves together with cocaine. The free base forms white shining plates melting at 49° . It dissolves with great difficulty in water but readily in alcohol, ether, chloroform and benzene. Aqueous solutions of tropacocaine have a strong alkaline reaction. As a rule salts of tropacocaine with one equivalent of acid crystallize well. The hydrochloride, $C_{15}H_{19}NO_2 \cdot HCl$, is used in medicine. Solutions of tropacocaine are optically inactive. Heated with concentrated hydrochloric acid, tropacocaine is decomposed into benzoic acid and pseudo-tropeine:



Pseudo-tropeine is stereo-isomeric with tropine. It melts at 108° and dissolves readily in water and alcohol but with more difficulty

in ether. Tropine melts at 63° and dissolves easily in water, alcohol and ether. Both are strong bases and boil without decomposition.

Reactions and Detection

In many respects tropacocaine is much like cocaine. Sodium hydroxide solution added to solutions of its salts causes milkiness and precipitates the amorphous base easily soluble in ether. Consequently in the Stas-Otto process tropacocaine will appear in the ether extract of the aqueous solution after it has been made alkaline with sodium hydroxide solution. Evaporation of the ether solution leaves tropacocaine as an oily residue. If the latter is warmed upon the water-bath for a short time to expel moisture, it becomes crystalline as it cools. Most of the general alkaloidal reagents added to solutions of tropacocaine salts produce abundant precipitates. More than traces of tropacocaine may be identified by some of its well crystallized salts and thus distinguished from cocaine. Tropacocaine hydrochloride crystallizes in leaflets melting with decomposition at $276-277^{\circ}$, whereas cocaine hydrochloride heated slowly melts sharply at $182-183^{\circ}$.

Saturated picric acid solution forms a difficultly soluble precipitate crystallizing from hot water containing a little alcohol in needles and leaflets that darken at $215-220^{\circ}$ and melt at $240-242^{\circ}$. Cocaine picrate melts at $165-166^{\circ}$.

Tropacocaine chloraurate, $C_{16}H_{19}NO_2 \cdot AuCl_4$, is formed even in extreme dilution and crystallizes from hot water without decomposition in yellow needles melting at 208° with decomposition, whereas cocaine chloraurate, $C_{17}H_{21}NO_2 \cdot AuCl_4$, is obtained in the form of a light yellow amorphous precipitate from which metallic gold easily separates.

Potassium dichromate, added to a 0.5 per cent. solution of tropacocaine hydrochloride, at once produces a thick crystalline precipitate that dissolves with some difficulty upon heating the liquid and again separates in compact crystals as the solution cools. The same reagent added to the corresponding cocaine solution produces only after acidification with a little hydrochloric acid a resinous precipitate that coalesces in oily drops when the liquid is warmed. As the liquid cools it again separates resinous and becomes finely crystalline only after long standing, usually after several days.

Physiological and Pharmacological Properties

The physiological behavior of tropacocaine is similar to that of cocaine. In anaesthetic action it is more rapid than cocaine and also less toxic. Anaesthesia from tropacocaine, however, is not as prolonged as that from cocaine. According to Kochmann,¹ the toxicity of tropacocaine is 8 times less than that of cocaine. In case of tropacocaine the ratio of local anaesthetic power to toxicity is more favorable than in that of cocaine. Small doses cause no enlargement of the pupil and even when it can be detected is very slight. Tropacocaine gives rise to no disturbances of accommodation and causes no increase of blood-pressure, since it enlarges the blood-vessels. In consequence of its action in enlarging blood-vessels, tropacocaine is not adapted for use in conjunction with preparations of the suprarenal glands or with adrenaline. According to Braun,² tropacocaine is useful in several ways for purposes of local anaesthesia. When anaesthesia of long duration is not desired, tropacocaine on account of its non-toxicity and freedom from irritation is to be preferred to cocaine. In ophthalmological work a 5 per cent solution appears to be specially serviceable. It causes anaesthesia of the cornea and conjunctiva in a few seconds which is amply sufficient for the removal, for example, of foreign bodies. In this case tropacocaine is a better anaesthetic than cocaine, for it acts more quickly, leaves the cornea unchanged, and avoids intra-ocular rise of blood-pressure as well as mydriasis. Addition of salt to the tropacocaine solution lessens any tendency to cause enlargement of blood-vessels and reddening of the conjunctiva. Tropacocaine is especially useful in producing anaesthesia of the spinal cord. In anaesthesia of the lumbar region it is essential that the anaesthetic used exert as little toxic action as possible upon the nerve roots and their nuclei. Experiments upon animals have shown that of the substances in the group comprising cocaine and cocaine-substitutes tropacocaine comes nearest to fulfilling this requirement. Following intralumbar administration of tropacocaine, anaesthesia appears after 3-4 minutes and lasts as a rule for 45-60 minutes. The average dose is 0.5-0.6 gram of tropacocaine hydrochloride. Injurious effects upon heart, lungs and kidneys caused by lumbar anaesthesia from tropacocaine are unknown. The most common after-effect is headache in the frontal region or occiput which may be of long or short duration and appear immediately or in a few days after the drug has been used. Very large doses of tropacocaine cause rise of temperature and outbreak of convulsions. Death ensues from respiratory paralysis (Kobert).

LOBELINE

Lobelia inflata, indigenous to North America, contains several active alkaloids that can be isolated from the plant by extraction with alcohol. Neutralization of the residue from this extract with acid, usually dilute sulphuric acid, converts it into a brown-yellow resinous mixture of salts known commercially as "Lobeline." The effects of very small subcutaneous doses of this lobeline recall apomorphine (central emesis), those from small internal doses and from smoking the leaves of *Lobelia inflata* recall nicotine, and those from large doses recall

¹ M. Kochmann. On Anaesthetics. *Therapeutische Monatshefte* 1914.

² H. Braun. Local Anesthesia, Its Scientific Foundations and Practical Application. Third Edition.

conune, since curare-like paralysis appears in frogs. In man the peripheral ends of the lung vagus are paralyzed by lobeline. Consequently asthmatic contractions of the bronchiae are allayed. For this reason official tincture of lobelia is specific for asthmatic pains. A dose of 11 mg of lobeline resulted in marked somnolence and diarrhoea. Vomiting did not appear. Following administration of large doses of lobeline to dogs and cats, muscular twitching and tonic convulsions appeared. Death ensued from paralysis of the respiratory centre.

Wieland¹ has isolated several alkaloids in pure crystalline form from *Lobelia inflata*. The most important compound is known as lobeline. To distinguish this product from commercial lobeline, the author suggests that this pure preparation be called "Lobelinum crystallisatum Wieland". Pure lobeline has the composition $C_{23}H_{29}NO_2$. It is a monacid base that appears in beautiful colorless crystals, melting at $128-129^\circ$, and forms well crystallized salts having a neutral reaction. Wieland has ascertained the following facts with regard to the constitution of lobeline. Quite probably the two oxygen atoms are in ether-like combination, since reagents for carbonyl and hydroxyl give no evidence of reacting. Methoxyl groups are not present. A lactone group also is probably not present, since lobeline is stable in presence of solutions of caustic alkalis. Heated with water, lobeline is decomposed into acetophenone, isolated to the extent of more than 60 per cent. of the weight calculated for one molecule, and a basic product having the composition $C_{16}H_{21}NO$ or $C_{15}H_{19}NO$.

The observation of Wieland that chloroform extracts lobeline hydrochloride from aqueous solution, while leaving the resinous salts of the other bases dissolved in water, is the basis of the isolation of crystallized lobeline. Faintly acidify the solution of the crude lobelia alkaloids with hydrochloric acid and make about 10 extractions, shaking vigorously each time with $\frac{1}{2}$ the volume of chloroform. Evaporation of the combined chloroform extracts, finally *in vacuo*, leaves a light brown syrup. Repeatedly digest the latter with water at 60° for 10 minutes each time. By allowing the filtered extracts to stand in a vacuum desiccator over solid potassium hydroxide and concentrated sulphuric acid, the hydrochloride is obtained in crystals.

The free lobeline base may be obtained by quickly dissolving the combined residues from crystallization in warm water, thoroughly cooling the solution, and decomposing lobeline hydrochloride in a separating funnel under ether with a slight excess of sodium hydroxide solution. Ether at once dissolves the free base. Dry the ether solution with potash, distil off most of the ether, and allow the concentrated solution to evaporate slowly. Crystals soon begin to appear. The crude base may be recrystallized from alcohol, benzene or ether. Pure lobeline dissolves in water and petroleum ether with great difficulty but is freely soluble in chloroform, hot benzene and hot alcohol. A cold saturated alcoholic solution contains 3.7 per cent of alkaloid. Lobeline is only slightly soluble in ether.

Lobelidine, $C_{26}H_{35}NO_3$, melting at 106° , is another crystalline lobelia alkaloid obtained by Wieland. In appearance pure lobelidine is not unlike lobeline but widely different from it in composition, melting-point and its hydrochloride.

¹ H. Wieland: Alkaloids of Lobelia I. Berichte d. Deutsch. chem. Ges. 54 (1921), 1784.

Detection

Since lobeline is a strong base, it is obtained in the Stas-Otto process when the aqueous solution made alkaline with sodium hydroxide or sodium carbonate solution is extracted with ether. Evaporation of solvent leaves an amorphous residue having a faint odor of the lobelia plant, provided poisoning was due to the plant. In this case the solution of this residue in water containing acid gives precipitates with potassium mercuric iodide, potassium bismuthous iodide, and phospho-molybdic acid. From solutions of lobeline salts, mercuric chloride precipitates difficultly soluble amorphous double salts (Wieland).

Impure lobeline, obtained from material after poisoning due to the plant or tincture, gives with Froehde's reagent after about 2 minutes a violet color that increases in intensity during a period of 1-2 hours and later changes to brown or yellow (Gadamer).

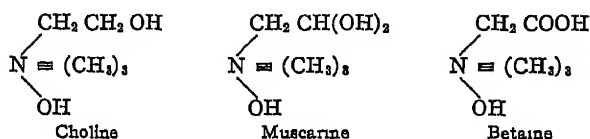
Behavior of Pure Lobeline toward Reagents

Lobeline dissolves in concentrated sulphuric acid without color. A very faint reddish color gradually appears and later becomes faintly brownish. Solution in concentrated nitric acid is also colorless. Marquis' reagent gives a deep violet to cherry-red solution. These colors appear immediately and are very stable. Froehde's reagent first gives a nearly colorless solution that gradually changes to faint rose-red and later has a greenish brown border. Mecke's reagent behaves like concentrated sulphuric acid. Mandelin's reagent gives a not very characteristic brown color with violet streaks and later becomes greenish. Picric acid gives a yellow crystalline precipitate that may be recrystallized from hot water. Picrolonic acid gives amorphous greasy precipitates. Chloro-platinic acid gives a light yellow precipitate consisting of small microscopic prisms. Potassium bismuthous iodide gives a precipitate consisting of compact red-brown crystals. Iodic acid is not reduced.

Wieland brings about the cleavage of acetophenone ($\text{C}_6\text{H}_5\text{CO.CH}_3$) by heating 2 grams of pure lobeline with 20 cc of water for 6 hours at 110° in sealed tube. The contents of the tube are dissolved in ether and the ether solution extracted with dilute hydrochloric acid to remove basic substances. The ether is distilled and the residue subjected to steam distillation. Acetophenone passes over leaving almost no residue. The distilled acetophenone is again dissolved in ether and finally weighed in a tared flask. The yield is 0.43 gram = 60 per cent of the quantity theoretically possible for the cleavage of 1 molecule of acetophenone. The loss is to be attributed to volatilization. For identification acetophenone was converted by an alcoholic solution of o-nitro-phenyl-hydrazine into its o-nitro-phenyl-hydrazone, $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{C} \\ \diagdown \\ \text{C}_6\text{H}_5 \end{array} = \text{N}-\text{NH}.\text{C}_6\text{H}_4.\text{NO}_2$, that crystallizes in beautiful orange-red needles melting at 143° .

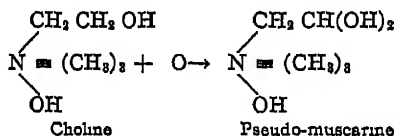
MUSCARINE

Muscarine, or oxycholine, $\text{C}_{10}\text{H}_{18}\text{NO}_2$, is closely related chemically to choline and betaine which are fairly widespread in the plant kingdom.



Muscarine was discovered in 1869 by Schmiedeberg and Koppe¹ in the fly mushroom, *Amanita muscaria*, together with other substances, particularly choline. To distinguish the natural product from other synthetically prepared muscarines, it is given the more precise designation "fly mushroom muscarine." That the latter is formed in the fly mushroom as a result of the oxidation of the abundant choline appears to be not entirely improbable. The statement made by different observers that all fly mushrooms do not contain muscarine is worthy of note. Northern fly mushrooms are said not to contain preformed muscarine. At least Nencki and Berlinerblau failed to find muscarine in the alcoholic extract of Siberian fly mushroom. It is first formed when the mushroom is boiled with water. The presence of muscarine, as shown by Brieger, in putrid codfish deserves mention. In this case also the alkaloid probably comes from choline always present in the flesh of fish. Muscarine also occurs in other mushrooms, such as the Panther mushroom, *Amanita pantherina*, *Boletus luridus*, and *Russula emetica*, according to R. Kobert.

Properties.—As usually obtained, muscarine is an inodorous, tasteless syrup without color when pure. It is deliquescent in air and has a strong alkaline reaction. It dissolves easily in water and alcohol but is nearly insoluble in ether and chloroform. Consequently muscarine cannot be extracted in the Stas-Otto process. Moreover it cannot be extracted from aqueous solution by benzene, benzene, ligroin or petroleum ether. Muscarine and its salts when warmed with potassium hydroxide solution give off trimethylamine, $\text{N}(\text{CH}_3)_3$, recognized by its fish-like odor. Choline, neurine and neuridine behave in the same way. A base containing one more atom of oxygen is formed by oxidation of choline, that is, by boiling its hydrochloride or its chloro-platinate with nitric acid. This compound has the same chemical composition as muscarine from fly mushroom but different physiological properties and consequently cannot be identical with the latter. This synthetic muscarine is called choline-muscarine or pseudo-muscarine.



Muscarine is characterized by its physiological action and by the appearance of its chloro-platinate, $(\text{C}_{12}\text{H}_{14}\text{NO}_3)_2\text{PtCl}_6 \cdot 2\text{H}_2\text{O}$, as difficultly soluble octahedrons or yellow needles and of its chlor-aurate, $\text{C}_{12}\text{H}_{14}\text{NO}_3 \cdot \text{AuCl}_4$, difficultly soluble in cold water.

Physiological Properties.—The most striking symptom of the action of pure muscarine is its actual or apparent irritation of the cardio-inhibitory center.

¹ O. Schmiedeberg and R. Koppe. Muscarine, the Poisonous Alkaloid of *Amanita Muscaria*. Leipzig, 1869.

sufficiently strong to produce complete stasis of the heart in no wise paralyzed. Many pharmacologists hold the view that muscarine by its action upon the heart shows itself to be a pure muscle poison causing paralysis. This stasis of the heart in diastole may cause death at once in man and mammals. Straub has furnished evidence that the heart is rendered incapable of functioning only when muscarine has penetrated the muscle fibres. The action of atropine upon the vertebrate heart is said to depend upon the fact that it diminishes the speed with which muscarine collects. Fungus muscarine stimulates all vertebrate peripheral apparatus and organs paralyzed by atropine. Consequently atropine almost instantly allays in vertebrates the effects of fungus muscarine. Previous administration of atropine will prevent appearance of the effects of muscarine. Atropine in its action is therefore markedly antagonistic to that of muscarine. The statements made by Schmiedeberg with regard to the symptoms produced by very small doses of muscarine are: lower frequency of pulse, fall of blood-pressure, salivation, flow of tears, increased pancreatic, biliary, mucous, spermatric and sweat secretion, contraction of pupils, accommodation spasms, and violent contractions of stomach and intestinal tract resulting in emesis and diarrhoea. In consequence of profuse secretion of intestinal glands stools may be watery and in the end bloody. As a result of the irritation of all glands the blood is thickened as in cholera and consequently the number of red blood-corpuscles in a unit volume of blood shows a marked increase. Finally contraction of bladder and uterus may occur. The effects in man following subcutaneous injection of 1-3 mg. of muscarine are: flow of saliva, rush of blood to the head, hence flushing of the face, increase in pulse frequency, nausea, rumbling in the abdomen, usually contraction of pupils, disturbances of vision, especially accommodation spasms, profuse perspiration on the face, and to a less degree on the rest of the body. In larger doses muscarine causes slowing of the pulse, vomiting, diarrhoea, convulsions, unconsciousness, and death due primarily to stasis of the heart (Kobert).

Use of the fly mushroom in northern lands as a beverage is of interest, that is, as a means of intoxication. Used for this purpose this fungus is said to produce typical intoxication accompanied by a condition of exhilaration and dilatation of pupils. Flow of saliva, emesis and diarrhoea, and pronounced slowing of the pulse are entirely lacking. A notable fact in this case is that subcutaneous injection of atropine, which easily allays all effects of intoxication arising from muscarine, does not end intoxication caused by drinking the beverage made from fly mushroom.

Detection and Isolation of Muscarine

Muscarine is quantitatively precipitated by mercuric chloride and potassium bismuthous iodide. Potassium mercuric iodide causes precipitation only when the reagent is added carefully and without excess of potassium iodide. Bromine water, chlor-auric acid, tannic acid, phospho-molybdic acid and phospho-tungstic acid also precipitate muscarine. The chlor-aurate and chloro-platinate of muscarine which crystallize beautifully probably suffice to characterize this base chemically (see above).

The method used by Brieger¹ in examining cadaveric material for ptomaines is especially adapted for isolating muscarine from organs and other parts of the cadaver. The material is comminuted and boiled with water containing a little dilute acid. The water should always show an acid reaction and boiling should not last longer than a few minutes. Evaporate the hot filtered solution upon the water-bath to a thick syrup. Gradually stir into the latter a considerable quantity of 96 per cent alcohol. Many substances such as protein bodies and inorganic salts remain undissolved. Filter this solution and while it is still warm add an alcoholic solution of lead acetate avoiding an excess. This lead precipitate contains no bases and is filtered off. Evaporate the filtrate to a syrup and again take this up in 96 per cent alcohol. Remove alcohol from this solution by evaporation or distillation. Dissolve the residue in water and remove lead by hydrogen sulphide. Warm to expel excess of hydrogen sulphide from the filtrate and precipitate with potassium mercuric iodide saturated with mercuric iodide. Most of the muscarine that may be present is precipitated. Filter off the precipitate, triturate with barium hydroxide, and decompose with hydrogen sulphide. Free the filtrate from barium with sulphuric acid and, having expelled hydrogen sulphide, remove iodine with silver chloride. The filtrate thus obtained after concentration is treated with chloro-platonic acid. Upon slow evaporation muscarine chloro-platinate crystallizes in octahedrons and needles that may be separated mechanically from red-yellow monoclinic plates of choline chloro-platinate. Evaporation of muscarine chloro-platinate solution with potassium chloride solution and extraction with alcohol yields muscarine hydrochloride, the alcoholic solution of which is evaporated. The residue taken up in water may be used for the physiological detection of muscarine.

Like potassium salts, for example, muscarine causes diastolic stasis of the frog's heart and is characterized by the fact that it can be counteracted by injection of atropine. In systolic stasis caused by substances acting like digitalis, the ventricle of the heart in consequence of maximal contraction appears pale, whereas in diastolic stasis at maximal expansion it appears dark. Addition of a drop of about 0.5 per cent atropine sulphate solution to the ventricle brought to stasis by muscarine soon causes reappearance of regular heart-beats and after that the action of the heart appears normal as before. Consult H. Fühner "Nachweis und Bestimmung der Gifte auf biologischem Wege (1911), page 77 (Biological Detection and Estimation of Poisons).

•Notes.—J. Gadamer ("Chemische Toxicologie") precipitates the faintly acid alcoholic extract of material, freed from alcohol, by lead acetate and ammonia in slight excess instead of an alcoholic lead acetate solution.

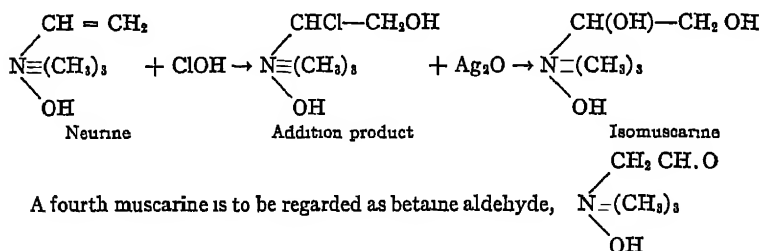
Synthetic Muscarines

Pseudo-muscarine, or muscarine from choline (see above), obtained by Schmiedeberg and Harnack² by oxidizing choline, has the same chemical composition as muscarine from fly mushroom but a different physiological action. It is there-

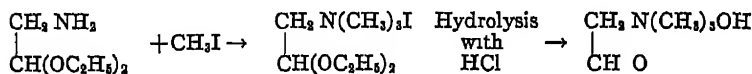
¹ L. Brieger Researches upon Ptomaines, III Part. Berlin 1886

² O. Schmiedeberg and E. Harnack. Synthesis of Muscarine and Muscarine-like Acting Ammonium Bases. Archiv f experim Pathol u Pharmac 6 (1877), 101.

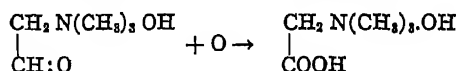
fore not identical with the latter Isomuscarine of Bode and Schmidt¹ has strong toxic properties which are widely different from those of muscarine from fly mushroom. The constitution of isomuscarine is shown by its formation from neurine by the action of moist silver oxide upon its addition-product with hypochlorous acid



It was first obtained by Berlinerblau² from chlor-acetal and trimethylamine. Later E Fischer methylated acetalamine and produced the corresponding aldehyde by hydrolysis of acetal-trimethyl-ammonium chloride with warm concentrated hydrochloric acid



This aldehyde-ammonium base is not identical with muscarine. Its physiological action, according to Schmiedeberg, is entirely different from that of muscarine from fly mushroom and is more suggestive of that of choline. In conformity with its structure as betaine aldehyde, E Fischer³ succeeded in oxidizing this substance with silver oxide and obtaining the carboxylic acid betaine



According to Nothnagel,⁴ betaine aldehyde, which may be regarded as anhydro-muscarine, in quantities up to 0.01 gram has not the slightest effect upon the action of the frog's heart. This base also has no action upon the cat's eye nor upon the vagus apparatus controlling arrestment of action of the mammalian heart. On the other hand, anhydro-muscarine like most ammonium bases gives rise to secretion of saliva and sweat in great abundance. Death in mammals is brought about by paralysis of respiration.

¹ E Schmidt Choline, Neurine and Related Compounds. *Annalen der Chemie* 267 (1892), 249. J. Bode. Certain Derivatives of Neurine and Choline. *Ibid.* 267 (1892), 268.

² J. Berlinerblau: Muscarine. *Berichte d. Deutsch. chem. Ges.* 17 (1884), 1139.

³ E Fischer. *Amino-Acetaldehyde*. *Ibid.* 26 (1893), 464 and 27 (1894), 165.

⁴ G. Nothnagel. Muscarine. *Ibid.* 26 (1893), 801.

DERIVATIVES OF MORPHINE

(Dionine, Heroin, Oxydimorphine)

DIONINE

Dionine, or ethyl-morphine hydrochloride, $C_{17}H_{17}O(OH)(OC_2H_5)N \cdot HCl \cdot H_2O$, a substitute for codeine, is a white, odorless, micro-crystalline powder having a bitter taste and melting at $123-125^\circ$. It dissolves easily in water and alcohol with neutral reaction but is insoluble in ether and chloroform. Most of the alkaloidal reagents precipitate dionine from pure aqueous solutions even in extreme dilution. In its color reactions dionine differs hardly at all from codeine. Slight differences appear only in its behavior toward ammonia. Free codeine, precipitated by a few drops of ammonia (sp gr 0.91 = 25 per cent) from 5 cc of a 10 per cent solution of codeine hydrochloride, remains permanently dissolved upon further addition of 1 cc of ammonia. Free ethyl-morphine base precipitated under the same conditions is first temporarily dissolved by 5 cc of ammonia and after a short time again separates in crystalline form. Free ethyl-morphine, $C_{17}H_{17}O(OH)(OC_2H_5)N \cdot H_2O$, melts at 93° and crystallizes in shining prisms readily soluble in alcohol, ether and chloroform, less easily in benzene, and soluble in 280 parts of water.

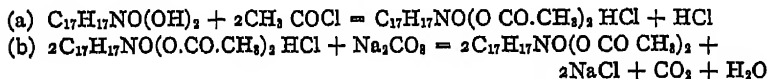
Marquis' reagent first colors dionine distinctly green, then blue, and finally a fine blue-violet. Mecke's reagent at once colors it olive-green. This color upon gentle warming changes, sometimes through blue, to blue-green. The same reactions that distinguish codeine from morphine apply to dionine, especially its great solubility in ether and chloroform.

Dionine has proved efficient as an anodyne and soporific.

HEROINE

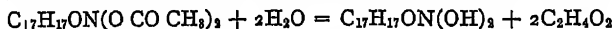
Heroin, or diacetyl-morphine, $C_{17}H_{17}ON(O \cdot CO \cdot CH_3)_2$, finds use as the free base and particularly in the form of the hydrochloride, $C_{17}H_{17}O(O \cdot CO \cdot CH_3)_2N \cdot HCl$, as a substitute for codeine on account of its specific sedative action upon the respiratory organs. It is, however, more toxic than morphine.

Preparation.—Heroin is prepared by heating finely powdered anhydrous morphine with acetyl chloride upon the water-bath. From the hydrochloride thus formed the pure base is precipitated by sodium carbonate and recrystallized from acetic ether:



Free heroin base crystallizes in white prisms having an alkaline reaction and a faintly bitter taste. It melts at $176-178^\circ$, is almost insoluble in water, difficultly soluble in ether and cold alcohol, and easily soluble in hot alcohol, chloroform and benzene. Caustic alkalis, ammonia and alkaline carbonates precipitate the base from its salts. Excess of caustic alkali redissolves heroin. When

boiled with water or dilute acids, but especially easily with sodium hydroxide solution, heroine readily undergoes hydrolysis into morphine and acetic acid



In consequence of this behavior a part of the heroine at least will be recovered as morphine in an examination following the Stas-Otto procedure. Therefore, if it is essential that heroine be detected as such, the material to be examined should be made faintly alkaline with sodium carbonate and extracted at once with ether, better with chloroform, which dissolves free heroine base.

Detection

Unlike morphine, heroine gives no blue color with ferric chloride solution. In making this test, use an aqueous solution of neutral heroine hydrochloride. Excess of acid should not be present. Heroine does not reduce iodic acid solution. Moreover in distinction from morphine heroine does not immediately color blue a mixture of potassium ferricyanide and ferric chloride solutions. A blue color appears only after long standing, when heroine has undergone partial hydrolysis. On the other hand, heroine gives all the morphine tests in which saponification can take place as is the case in all reactions involving concentrated sulphuric acid. For this reason heroine responds to the morphine tests with the reagents of Froehde, Husemann, Marquis, Mecke and Pellagri.

According to Zernik, the following two tests may be used to differentiate heroine from morphine.

(a) A trace of heroine rubbed with a few drops of concentrated nitric acid (sp. gr. 1.4 = 60 per cent) dissolves with yellow color. Extending from the centre of the drop to the margin, a green-blue color soon develops at ordinary temperature, but at once upon warming, and in time fades again to yellow.

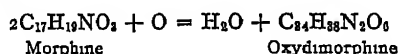
(b) **Detection of Acetyl Groups.**—If a little alcohol is added to a trace of heroine, that has been heated with sulphuric acid, and the mixture is warmed, the odor of acetic ether may be detected. Obviously a positive result with this test is proof of the presence of heroine only in conjunction with the above morphine tests.

Heroine is considerably more toxic than morphine and, inasmuch as medicinal doses 0.003–0.01 gram are very close to the toxic dose, heroine has repeatedly been the cause of poisonings with fatal termination.

OXYDIMORPHINE

Oxydimorphine, or pseudo-morphine, $\text{C}_{24}\text{H}_{38}\text{N}_2\text{O}_6$, occurs in opium in small quantity and deserves attention since it appears in urine probably as a decomposition product of morphine in the human organism following administration of morphine. Dragendorff and other of the older authors assume "that most of the morphine is changed into inactive oxydimorphine or pseudo-morphine." The above assumption is easily explained in view of the fact that morphine, especially in alkaline solution, is converted into oxydimorphine by atmospheric oxygen and most of the oxidizing agents,

such as potassium permanganate, ammoniacal copper solution, potassium ferricyanide solution and other compounds of similar nature. But at the present time the author does not know of a case where pure morphine-free oxydimorphine has been obtained from the urine of persons poisoned by morphine or from that of morphine addicts



Oxydimorphine is not toxic. Nascent hydrogen does not reconvert it into morphine. Oxydimorphine forms a white, crystalline powder almost insoluble in water, alcohol, ether and chloroform. Caustic alkalis precipitate it from solutions of its salts as a fine white powder soluble in excess of precipitant. Ammonia also liberates the base but redissolves it only when present in great excess. Strongly ammoniacal isobutyl alcohol, according to R. Kobert, is well suited for the extraction of oxydimorphine.

Detection

Oxydimorphine in most of its reactions shows a very strong resemblance to morphine, for example, in its behavior toward ferric chloride, iodic acid, concentrated nitric acid, and Froehde's reagent. Gadammer points out the following differences between the two alkaloids:

With Marquis' reagent oxydimorphine gives an intense yellow-red color; morphine a violet-blue color.

With concentrated sulphuric acid and cane-sugar oxydimorphine gives a blue color that gradually changes to green; morphine a red color.

With 8 drops of concentrated sulphuric acid 1 mg. of oxydimorphine gives a yellow solution; morphine a colorless solution. Upon gentle warming an intense green color appears; morphine gives a reddish color. After cooling, addition of 10 drops of water produces a rose color. If the liquid is washed into a test-tube with 10 drops of water and about 40 drops of water are added until the color disappears, the liquid becomes turbid from separation of oxydimorphine sulphate. If the liquid is now divided into 3 parts and 10-20 drops of concentrated nitric acid are carefully added to one part, 1 drop of sodium nitrite solution (5 per cent.) to the second part, and 1 drop of sodium hypochlorite solution to the third part, a dark violet color will appear in each solution, provided oxydimorphine is present. Morphine gives a red color.

Furthermore a trace of oxydimorphine and 2 drops of sodium hypochlorite solution rubbed together upon a watch-glass instantaneously produce a saffron-yellow color quickly changing to pale yellow. This mixture, if then stirred with 8 drops of concentrated sulphuric acid, takes on a fine emerald-green color. Morphine undergoes only a slight change, whereas codeine gives a sky-blue color

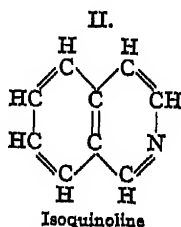
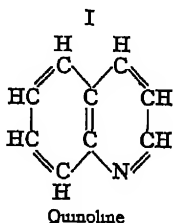
PAPAVERINE

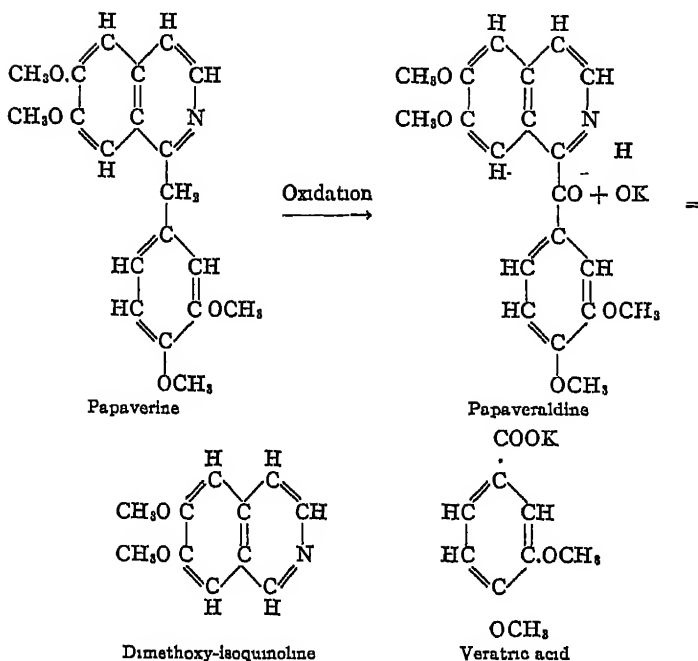
Papaverine, $C_{20}H_{21}NO_4$, constitutes about 0.5-1 per cent of opium. When crude it is usually mixed with narcotine. To remove the latter, prepare the acid oxalate of papaverine which dissolves with difficulty in water. Crystallize this salt from boiling water until it dissolves in concentrated sulphuric acid without color. Convert papaverine oxalate into the hydrochloride by treatment with calcium chloride and then liberate the alkaloid with ammonia. This product crystallized from alcohol is pure papaverine.

Papaverine crystallizes in fine colorless prisms melting at 147° . This alkaloid is insoluble in water, soluble with difficulty in ether (1:250), cold alcohol and benzene, but freely soluble in hot alcohol, acetone and chloroform. These solutions are neutral, not bitter, and optically inactive. Papaverine is a weak base which dissolves in but does not neutralize acetic acid. Ether partially extracts it from an aqueous tartaric acid solution and completely extracts it from alkaline solution. Consequently this alkaloid appears in the Stas-Otto process in ether extract B. Chloroform extracts papaverine with almost as much ease from acid as from alkaline solution.

Constitution—Papaverine is a monacid, tertiary base that combines with 1 molecule of an alkyl iodide to form crystalline addition products. As it forms no acetyl derivative with acetic anhydride, free hydroxyl is not present. But there are probably four methoxyl groups, for it loses four methyl groups when treated with hydriodic acid according to Zeisel's method. Consequently all oxygen atoms in papaverine are present as methoxyl groups. The researches of Goldschmiedt, extending from 1883 to 1898, have completely explained the constitution of papaverine. Moderate oxidation with potassium permanganate and sulphuric acid gives papaveraldine, $C_{20}H_{19}NO_3$, without breaking the carbon chain. Fusion with potassium hydroxide breaks the latter into nitrogen-free veratric acid and the nitrogenous base dimethoxy-isoquinoline:¹

¹ Isoquinoline (II) is isomeric with quinoline (I) and like the latter is a monacid, tertiary base.





Detection of Papaverine

The following general reagents precipitate papaverine in a dilution of 1:10,000: iodo-potassium iodide, phospho-molybdic acid and potassium bismuthous iodide; and in a dilution of 1:5000 tannic acid, gold chloride and potassium mercuric iodide give precipitates.

Special Reactions

1. **Concentrated Sulphuric Acid.**—A cold colorless solution of papaverine in this acid becomes dark violet upon gentle warming. But even a cold solution of impure papaverine in this acid is violet.

2. **Froehde's Test.**—Pure papaverine dissolves in this reagent without color. On the other hand, the commercial product gives a violet color gradually changing to blue-green, green and yellow.

3. **L. E. Warren's Test.**—Crush a very small crystal of potassium permanganate with a glass rod and intimately mix about 0.0005 gram of papaverine with the powder. Stir this mixture into about 0.2 cc. of Marquis' reagent. A green color, almost instantly changing to blue, appears. The latter color deepens into an intense violet-

blue which after some time becomes bluish green, green and finally dirty brown.

Of thirty-nine alkaloids tested, the only one in any way simulating papaverine was an unnamed alkaloid separated from *sanguinaria*

4. Concentrated Nitric Acid.—This acid as well as Erdmann's reagent dissolves pure papaverine without color. Heat to boiling a solution of 1 part of papaverine with 10 parts of nitric acid (sp. gr. 1.06 = 10 per cent HNO_3). As the solution cools, yellow crystals of the nitrate of nitro-papaverine, $\text{C}_{20}\text{H}_{20}(\text{NO}_2)\text{NO}_4 \cdot \text{HNO}_3 \cdot \text{H}_2\text{O}$, appear. Pale yellow prisms of nitro-papaverine, $\text{C}_{20}\text{H}_{20}(\text{NO}_2)\text{NO}_4 \cdot \text{H}_2\text{O}$, may be obtained from this nitrate by means of ammonia.

5. Mecke's Test.—This reagent dissolves pure papaverine in the cold with a greenish color which soon becomes dark steel-blue and finally deep violet. Upon warming the solution a dark violet color appears at once.

Physiological Action—According to Pal, papaverine lowers the tonus of smooth muscles but especially when increased through illness. Papaverine hydrochloride, used internally or subcutaneously in doses of 0.03–0.08 gram, or intravenously in doses from 0.01–0.02 gram, has proved a harmless and frequently a very active agent in the treatment of many vascular spasms. Frequently in doses of 0.005–0.05 gram several times daily it has proved quite useful in treating diarrhoea in children. It exerts a relaxing action upon the uterus. Its physiological action is similar to that of narcotine. In small doses papaverine has a narcotic action upon mammals, in larger doses it produces tremors, muscular tension and convulsions. In man after 0.18 gram of papaverine great languor and muscular weakness have been observed (Lewin). Following internal or subcutaneous administration, papaverine has been found in almost all organs, for example, the liver as well as in bile and urine.

THEBAINE

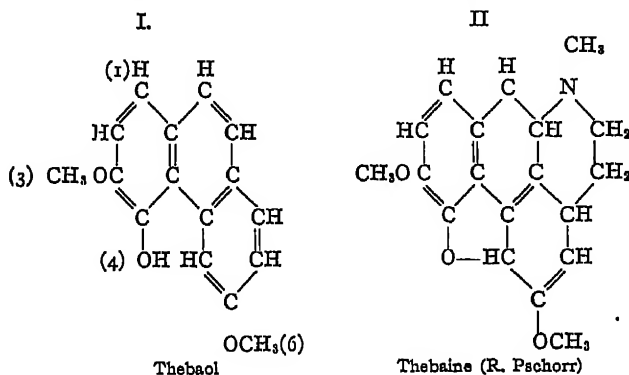
Thebaine, $\text{C}_{19}\text{H}_{21}\text{NO}_3$ or $\text{C}_{17}\text{H}_{15}(\text{OCH}_3)_2\text{NO}$, occurs in opium to the extent of 0.2–0.5 per cent. Crude commercial thebaine may be purified by solution in a little dilute acetic acid, decolorizing by means of animal charcoal, and then adding powdered tartaric acid. After long standing thebaine tartrate separates and is recrystallized from a little boiling water. Free thebaine is again precipitated by ammonia and finally recrystallized from hot alcohol. Thebaine may also be separated by means of sodium salicylate.

From hot dilute alcohol thebaine crystallizes in colorless leaflets resembling benzoic acid; from strong alcohol in thick prisms. It

melts at 193° . In cold water it is almost insoluble but easily soluble in alcohol, benzene and chloroform. It requires 140 parts of ether for solution. Solutions of thebaine are laevo-rotatory and have an alkaline reaction. Its taste is not bitter. Caustic alkalies, ammonia, milk of lime, sodium carbonate and bicarbonate precipitate the free base from solutions of its salts. The hydrochloride, salicylate and acid tartrate are crystalline compounds. Thebaine has scarcely any narcotic action and may be considered as a pure convulsive poison (von Schroeder).

Constitution.—Thebaine is a rather strong, monacid, tertiary base, forming as a rule well crystallized salts with acids. But excess of acid, especially mineral acid, usually decomposes these salts with ease. Being a tertiary base, it easily combines with methyl iodide forming thebaine iodomethylate, $C_{15}H_{21}NO_3 \cdot CH_3I$, crystallizing in prisms. Two of the three oxygen atoms in thebaine are methoxyl-groups ($-OCH_3$) and the third probably forms an ether-like combination, a so-called bridge-oxygen. The thebaine molecule appears not to contain hydroxyl. Therefore thebaine differs from morphine in not being soluble in sodium or potassium hydroxide solution.

Heated with acetic anhydride, thebaine gives the acetyl derivative of the phenol thebaol, $C_{15}H_{14}O_3$, and a nitrogenous product, methyl-oxy-ethylamine, $CH_3 \cdot NH \cdot CH_2 \cdot CH_2 \cdot OH$. R. Pschorr has synthesized thebaol, or the methyl ether of thebaol, and shown by this synthesis that thebaol is 3, 6-dimethoxy-4-oxy-phenanthrene (I). Pschorr assigns to thebaine the structural formula (II) which is analogous to that of apomorphine and morphine (see pages 220 and 227).



Detection of Thebaine

Ether and chloroform extract thebaine from alkaline aqueous solution and consequently this alkaloid appears in ether extract B, if the Stas-Otto procedure is followed. The general reagents,

phospho-tungstic acid, iodo-potassium iodide, potassium mercuric iodide and potassium bismuthous iodide precipitate thebaine even from very dilute solutions. Thebaine gives the following color reactions:

1. **Concentrated Sulphuric Acid.**—Thebaine dissolves in this acid with a deep red to blood-red color that gradually becomes yellowish red. The reagents of Erdmann, Froehde and Mandelin give the same result.

2. **Concentrated Nitric Acid.**—Thebaine dissolves in this acid with a yellow color.

3. **Mecke's Test.**—In the cold thebaine dissolves in this reagent with a deep orange-red color that soon fades and with heat becomes dark brown.

4. **Marquis' Test.**—Thebaine dissolves in this reagent with a yellow-red to brown color.

THEOBROMINE

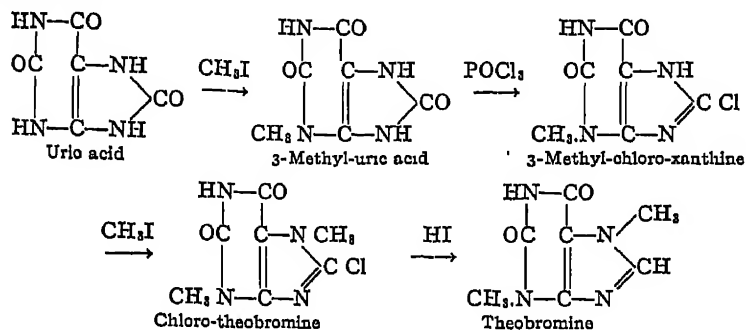
Theobromine or 3,7-dimethyl-xanthine, $C_7H_8N_4O_2(CH_3)_2$, is a constituent of cacao beans, occurring in the cotyledons to the extent of 1.4–1.8 per cent, whereas the hulls contain only 0.5–1.3 per cent. Young, air-dried cacao leaves also contain as high as 0.55 per cent of theobromine, whereas old leaves contain almost none. Cola nut and guarana paste contain only small quantities of theobromine together with a relatively large quantity of caffeine.

Properties.—Theobromine is a bitter, white powder consisting of microscopic needles. It sublimes almost without decomposition when carefully heated. The part of theobromine dissolves in 3280 parts of water at 18° and in 149 parts at 100° in 1500 parts of 90 per cent alcohol at 15°, in 3845 parts of acetic ether and 1000 parts of boiling chloroform. It dissolves in 4284 parts of cold absolute alcohol and in 422 parts of boiling alcohol. It is almost insoluble in pure ether but easily soluble in phenol. Solutions of theobromine react neutral and are optically inactive. Theobromine manifests the properties of a weak base and at the same time those of a weak acid. Consequently it dissolves easily in sodium or potassium hydroxide solution, as well as in mineral acids, forming salts. Although salts of theobromine with acids crystallize, they are partially decomposed by water into theobromine and acid and also when heated to 100°, provided the acid is volatile. Theobromine hydrochloride, $C_7H_8N_4O_2.HCl.H_2O$, obtained by dissolving theobromine in hot concentrated hydrochloric acid, forms colorless needles. Of the salts formed with bases, that containing silver, $C_7H_7N_4O_2Ag.1.5H_2O$, deserves mention. It appears as a white crystalline powder almost insoluble in water, when a solution of theobromine in dilute ammonia is treated with silver nitrate and then boiled for some time.

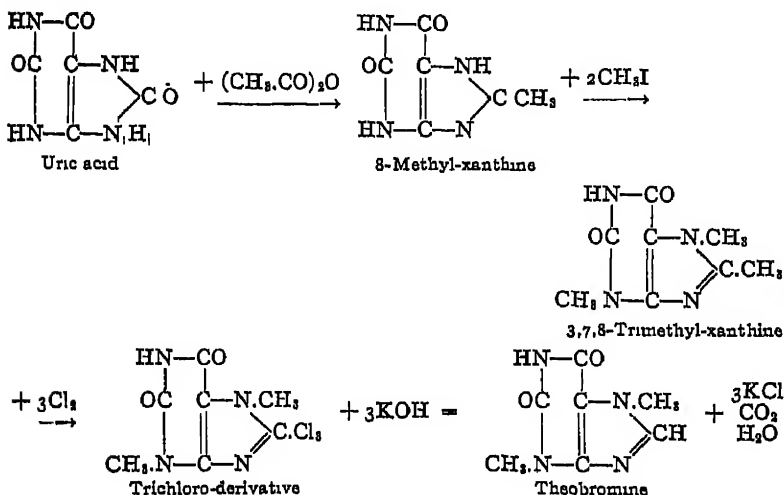
Synthetic Theobromine.—E Fischer obtained theobromine by heating lead xanthine for several hours with methyl iodide at 100°.



Upon the basis of this synthesis, theobromine is regarded as dimethyl-xanthine. E. Fischer also obtained theobromine by starting from uric acid. By means of methyl iodide at about 80° uric acid in alkaline solution was converted into 3-methyl-chloro-xanthine. The latter when methylated gave chloro-theobromine which was reduced by nascent hydrogen, that is, by hydriodic acid, to theobromine.



Technical Preparation.—Uric acid heated with acetic anhydride gives 8-methyl-xanthine. This compound in the form of its di-potassium salt heated with methyl iodide is converted into 3,7,8-trimethyl-xanthine. The vigorous action of chlorine upon the latter gives a trichloro-derivative from which through cleavage of carbon dioxide, water and potassium chloride by means of potassium hydroxide solution theobromine is obtained.



Physiological Properties.—Theobromine gives rise to nearly the same physiological effects given by the other purine derivatives of this series, caffeine and theophylline (see caffeine, page 147). It is an excellent diuretic. According to Schmiedeberg, however, in producing muscular rigidity its action is stronger than that of caffeine but it does not act as strongly as the latter in causing convulsions. Veley and Waller¹ allowed 0.02–0.003 n-caffeine and theobromine solutions in sodium chloride to act upon isolated muscle and determined how long a time elapsed before the muscle was not irritated. In these experiments the toxicity of theobromine to that of caffeine was in the ratio of 1 : 7 : 1. According to Kobert, theobromine exerts a specific irritating action upon the kidneys. But very likely the toxicity of theobromine is less than that of theophylline. Not until the daily dose of theobromine exceeds 2–5 grams is it said to cause vomiting, violent headache and albuminuria.

Detection of Theobromine

Amalic Acid Test.—If theobromine is quickly evaporated to dryness upon the water-bath, with about 100 times the quantity of chlorine water, or a solution of theobromine in dilute hydrochloric acid with some potassium chlorate, a red to red-brown residue remains. The latter brought in contact with a little ammonia gives a purple-violet color. This test may be made by covering the dish containing the residue with a glass plate previously moistened with a drop of strong ammonia. More explicit details concerning this test will be found under caffeine upon page 148.

Chloroform is the best solvent for extracting theobromine from an acidified solution. Gadamer gives the following directions for detecting theobromine. Dry the material thoroughly upon the water-bath or in a drying-closet, free it from fat by means of petroleum ether, and then extract under reflux with alcohol (80 per cent. by volume) faintly acidified with sulphuric acid. Remove alcohol from the filtered extracts by distillation or evaporation, neutralize the residue with magnesium oxide, dry and extract for a long time in a Soxhlet with chloroform. For purification stir the residue remaining after evaporation of chloroform with water containing sulphuric acid, then filter and again extract theobromine with hot chloroform. The solvent now leaves the purine base in a nearly pure condition. The residue may finally be subjected to sublimation between two watch-glasses. The distinctly crystalline sublimate deposited upon the upper watch-glass, in case theobromine is present, may be used for the amalic acid test.

¹ V. H. Veley and A. D. Waller. Relative Toxicity of Theobromine and Caffeine, as expressed in the Direct Action upon the Contractility of Isolated Muscle. Proc. Royal Soc. London. Series B. 82 (1910), 568.

Urine.—Theobromine like caffeine is also partially demethylated in the human organism so that urine contains 3- and 7-methyl-xanthine separated in part as such. Stomach-contents, vomitus, blood and urine are suitable material for examination. It is a notable fact that the three xanthine bases, caffeine, theophylline and theobromine, which find use in medicine as diuretics, are quite stable toward putrefaction.

Diuretine or Sodium-Theobromine-Sodium Salicylate

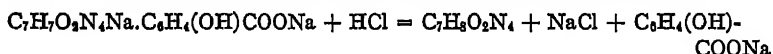
Theobromine dissolves in water with great difficulty. For this reason it is convenient to use the double compound diuretine which has an alkaline reaction and is soluble in water. Diuretine as well as the analogous agurine, theobromine-sodium acetate, are much more prone than caffeine to give rise to gastric and intestinal disturbances (H. Meyer-Gottlieb).

Preparation.—Dissolve theobromine in the calculated quantity of sodium hydroxide, adding the equivalent amount of sodium salicylate and evaporating to dryness, best *in vacuo*. Diuretine corresponding to the formula, $C_7H_7O_2N_4Na \cdot C_6H_4(OH)COONa$, is a white, odorless powder having a sweetish saline and somewhat alkaline taste. Its solutions in water have an alkaline reaction. It contains about 45 per cent of theobromine.

Estimation of Theobromine in Diuretine

Dissolve 2 grams of diuretine in 10 cc. of water, adding sufficient n-hydrochloric acid to produce a faint red color upon blue litmus paper. Then add a drop of ammonia, stir occasionally and allow the mixture to stand for 3 hours at ordinary temperature. Collect the theobromine that separates upon a weighed filter, or better in a weighed Gooch crucible, rinse twice using 10 cc. of cold water each time, dry at 100° and weigh. The content of theobromine in 2 grams of diuretine should be at least 0.8 gram.

Notes.—The quantity of hydrochloric acid added to the diuretine solution should not be more than is necessary to decompose sodium-theobromine.



Excess of hydrochloric acid would also decompose sodium salicylate causing separation of salicylic acid and in addition a small quantity of theobromine would be dissolved.

THEOPHYLLINE

Theophylline, theocine or 1,3-dimethyl-xanthine, $C_7H_8O_2N_4 \cdot H_2O$ or $C_5H_2O_2N_4(CH_3)_2 \cdot H_2O$, was first obtained by Kossel from the residues left after separation of caffeine from tea. Details of the synthesis of theophylline from malonic acid and dimethyl-urea are given under caffeine (see page 147). In that place the structural formula of theophylline is also discussed.

Theophylline forms thin monoclinic plates or needles melting at $264-265^{\circ}$. It dissolves easily in hot water and boiling alcohol but with difficulty in cold water and cold alcohol. Theophylline salts usually crystallize well. In accordance with its double character of base and acid, theophylline forms salt-like compounds both with acids and strong bases. Consequently this purine derivative is soluble in caustic alkalies as well as in acids.

Sodium-theophylline, $C_7H_7O_2N_4Na \cdot H_2O$, is a white crystalline powder soluble in water with strong alkaline reaction. It forms double salts corresponding to diuretine, for example, sodium-theophylline-sodium acetate, $C_7H_7O_2N_4Na \cdot C_2H_3O_2Na$, a white powder having an alkaline reaction.

Physiological Properties.—Theophylline exerts strong diuretic action and is very frequently used especially in dropsy. In cases where other diuretics have failed, frequently theophylline has given excellent results. But these results have often been attended with unpleasant secondary effects, for theophylline sometimes causes nausea, vomiting, epileptic convulsions, haemorrhages of the gastric mucosa and gastric ulcers. Experiments upon animals have shown that irritation of the kidneys is produced. As the maximal daily dose, 0.8 gram of pure theophylline is recommended.

Detection of Theophylline

Theophylline may be extracted from material by the same method given for theobromine. It may also be extracted by chloroform from an acidified solution and may be sublimed, best between two watch-glasses. The sublimate formed upon the upper watch-glass may be used for the amalic acid test described for theobromine (see page 414). Theophylline differs from caffeine and theobromine in that its solution in sodium hydroxide gives a red color with the diazo reagent.¹

TOXALBUMINS

Toxalbumins are toxic, protein-like substances that are either already formed in the plant or animal organism, or are produced in the metabolism of pathogenic micro-organisms. As yet these substances have not been isolated in a state of purity as individual chemical compounds. As a matter of fact, the chemical and physiological properties of such vegetable toxalbumins as abrin, ricin, robin and crotin are given by substances obtained from some particular part of the plant by a definite method. The vegetable toxalbumins

¹ See "Preparation of Reagents," page 641.

mentioned possess the common property of clumping, agglutinating and precipitating red blood-corpuscles. Therefore R. Kobert classifies them as "vegetable agglutinines." A trace of one of these agglutinines, added to defibrinated blood in a test-tube, causes clumping into a mass resembling sealing-wax. Abrin, ricin and crotin also cause coagulation of milk.

ABRIN

This toxalbumin occurs in jequirity seeds from *Abrus precatorius*. Remove the seed envelopes and extract the finely divided seeds with 4 per cent. sodium chloride solution. Concentrate the filtered liquid *in vacuo* and acidify with acetic acid. Precipitate abrin from this solution by addition of sodium chloride and finally purify by dialysis. Abrin is an amorphous, highly toxic powder not entirely free from ash. Though abrin and ricin are alike in some respects, they are not identical.

RICIN

This intensely toxic toxalbumin constitutes 2.8-3 per cent. of castor bean. Remove the seed envelopes and subject the seeds to powerful pressure to remove as much oil as possible. Then extract with 10 per cent. sodium chloride solution. Saturate the filtered extract at the same time with magnesium and sodium sulphate and keep for some time in the cold at room temperature. Place the precipitate containing ricin, in a parchment paper dialyzing tube and dialyze for several days. Finally dry the residual ricin *in vacuo* over sulphuric acid.

Ricin is an amorphous, highly toxic powder containing ash and easily soluble in 10 per cent. sodium chloride solution. This toxalbumin, dissolved in sodium chloride solution, gives the protein reactions. Ricin possesses in high degree the power of agglutinating blood-corpuscles, that is, the blood-corpuscles clump together leaving a clear serum. For this test-tube experiment use defibrinated blood, not diluted blood, nor blood mixed with physiological salt solution, that is, 0.9 per cent. solution. Ricin, according to Elfstrand, agglutinates red blood-corpuscles of the guinea-pig even in dilution of 1:600,000. Ricin agglutinates the blood of all mammals but not to the same degree. Removal of serum from blood and substitution of physiological salt solution strengthen rather than weaken the agglutinating action of ricin. The inference is that serum must

possess a certain anti-agglutinating action. Separation of red blood-corpuscles into stroma and haemoglobin¹ shows that ricin has not changed haemoglobin in the least. But the stromata in the same manner as the blood-corpuscles have been altered.

To detect ricin in castor bean press-cake, or in feeds containing castor beans, extract the finely divided material with physiological salt solution at room temperature, filter and make the agglutination test in a test-tube with undiluted, defibrinated blood and with blood diluted with physiological salt solution.

CROTIN

Crotin is a substance obtained from the seeds of *Croton Tiglium*. Remove the seed envelopes, express the oil and treat as described for abrin and ricin. Crotin chemically is quite similar to ricin. Abrin and ricin agglutinate blood-corpuscles of all warm-blooded animals thus far tested but crotin does not manifest the same behavior with all kinds of blood. (See R. Kobert's "Intoxikationen.")

Coagulation of Blood and Defibrinated Blood

Blood is a transparent fluid, the blood-plasma, suspended in which is a very large number of solid particles, red and white blood-corpuscles. Outside the organism blood coagulates even in a few minutes after being drawn. In the clotting of blood a very difficultly soluble protein called fibrin separates. If the blood is still, the clot is a solid mass that gradually contracts and exudes a clear liquid, usually yellow, the blood-serum. The coagulum, thus formed and enveloping the blood-corpuscles, is called the crassamentum (*Placenta sanguinis*). But if the blood is whipped during coagulation, fibrin separates in threads. The fluid separated from the latter is defibrinated blood consisting of blood-corpuscles and blood-serum. To obtain defibrinated blood, whip fresh blood removed from a vein with twigs and fibrin will separate upon the latter. Or run fresh blood into an Erlenmeyer flask containing iron filings and shake vigorously for several minutes. Fibrin is precipitated on the filings.

Coagulation of blood may be retarded by cooling suddenly to low temperature. By drawing the blood direct from the vein into a neutral salt solution and stirring, for example, magnesium sulphate solution (1 volume of salt solution + 3 volumes of blood), a blood-salt mixture that will remain uncoagulated for a day is obtained. Blood may be added to sufficient dilute potassium oxalate solution to give a mixture containing 0.1 per cent. of oxalate. Soluble calcium salts of the blood are precipitated by oxalate and blood loses its power of coagulating. To prepare a non-coagulating blood-plasma, pour blood into sodium fluoride

¹ Blood-corpuscles are made up of two principal components, namely, the stroma, which constitutes the true protoplasm, and the intraglobular contents, the chief constituent of which is haemoglobin.

solution until it contains 0.3 per cent of NaF. Aqueous solution of sodium citrate also retards coagulation of blood.

CYTISINE

Cytisine, $C_{11}H_{14}N_2O$, occurs in ripe seeds of Golden chain (*Cytisus Laburnum*) to the extent of about 1.5 per cent. Cytisine and the alkaloid originally called ulexine, isolated from seeds of *Ulex europaeus*, are identical (A. Partheil).

Cytisine crystallizes in large, colorless, inodorous prisms melting at 152° and subliming at higher temperature if carefully heated. It dissolves freely in water, alcohol, chloroform and acetic ether; less easily in commercial ether, benzene and acetone, and is almost insoluble in petroleum ether and absolute ether.

Cytisine is a strong secondary base and very toxic. Although capable of combining with 1 or 2 molecules of hydrochloric acid, this compound behaves in other respects like a monacid base. Only salts containing one equivalent of acid crystallize well. Nitrous acid converts this secondary base into nitroso-cytisine, $C_{11}H_{13}ON_2NO$, crystallizing in needles. Nitrous fumes appear, if cytisine is warmed upon the water-bath with twice the amount of concentrated nitric acid, and the solution at once becomes reddish yellow to brown. This solution poured into water gives a precipitate of nitro-nitroso-cytisine, $C_{11}H_{12}ON(NO_2)NNO$. This compound crystallizes from water in pale yellow scales melting at $242-244^\circ$. According to van de Moer¹ cytisine is an apopilocarpine.

Toxic Action.—Cytisine produces convulsions, its action in this respect being quite similar to that of strychnine. But unlike the latter alkaloid it also irritates the gastro-intestinal mucosa even causing bloody inflammation. Cytisine also differs from strychnine in stimulating the vomiting center. Consequently after doses of cytisine or laburnum preparations, human beings and animals capable of emesis thus rid the organism of a large part of the poison. Like strychnine, cytisine stimulates the respiratory and vaso-motor centers. Finally, as in strychnine intoxication, death results from paralysis of these two centers. A part of the cytisine leaves the organism unchanged and appears in the urine.

Detection of Cytisine

Prepare an aqueous tartaric acid solution of stomach-contents, vomitus or parts of organs, following the general procedure for alkaloids. To remove final traces of fatty acids and fat, shake this solution well with ether. Withdraw the aqueous solution, make alkaline with sodium hydroxide solution, and extract thoroughly with chloroform or better with isobutyl alcohol. Evaporate the chloroform or isobutyl alcohol extracts and test the residue as follows for cytisine.

1. **Van de Moer's² Test.**—Ferric chloride solution colors cytisine and its salts blood-red. Dilution with water, or acidification, discharges this color. Hydrogen peroxide also produces the same result. The solution containing hydrogen peroxide, warmed upon the water-bath, becomes intensely blue.

¹ J. van de Moer. *Synthesis of Cytisine*. *Nederl. Tijdschr. Pharm.* 7 (1895), 362.

² Ber. d. Deutsch. Pharm. Ges. 5 (1895), 267.

2. **Rauwerda's¹ Test.**—A little nitrobenzene, containing dinitro-thiophene, poured upon cytisine gives a fairly stable, red-violet color. A similar color given by conine is very unstable.

3. **Nitro-Nitroso-Cytisine Test.**—Nitro-nitroso-cytisine (see above), formed by concentrated nitric acid, serves to detect small quantities of this alkaloid. Nitro-nitroso-cytisine crystallizes from 94 per cent alcohol in compact prisms and from 50 per cent alcohol in flat plates. Water again precipitates it unchanged from solution in concentrated hydrochloric acid.

GLUCOSIDES AND SAPONIN SUBSTANCES

DIGITALIS GLUCOSIDES

The digitalis plant, *Digitalis purpurea* L., contains in all its parts, but especially in leaves and seeds, medicinally useful substances belonging to the glucoside group. Thus far three digitalis glucosides have been isolated as well characterized crystalline compounds of homogeneous composition. These are digitalin in the stricter sense, or *Digitalinum verum crystallisatum* Kiliani, $C_{36}H_{60}O_{14}$; digitoxin, $C_{44}H_{70}O_{14}$; and digitonin, $C_{66}H_{90}O_{29}$. A fourth glucoside called digitalein seems as yet not to have been obtained wholly pure.

DIGITOXIN

Digitoxin, $C_{44}H_{70}O_{14}$, occurs almost exclusively in digitalis leaves. This very active and highly toxic compound is almost entirely insoluble in water and ether but soluble in alcohol and chloroform. Consequently ether precipitates it from chloroform solution. Warmed with concentrated hydrochloric acid, digitoxin gives a green to brownish green color. According to Kiliani, it crystallizes from 85 per cent. alcohol in leaflets melting at 145° .

Pure digitoxin, according to the recent exhaustive investigation of Cloetta,² melts considerably higher, that is, sharply at $252-253^{\circ}$. It is obtained by dissolving the impure product in hot chloroform, adding ether to the solution while warm, and quickly filtering off the crystals before the solution has cooled. Once impurities have been removed, the melting-point 252° does not change, no matter whether this pure digitoxin is finally crystallized from alcohol or acetone with addition of water, or from chloroform with addition of

¹ A. Rauwerda. Contributions to the Knowledge of Cytisine and Its Alkyl-derivatives. Chem. Zentralbl. 1900 II. 268; Nederl. Tijdschr. Pharm. 12 (1900), 161.

² M. Cloetta. Contribution to the Knowledge and Pharmacology of Digitoxin and Its Cleavage-products. Arch. f. exper. Path. u. Pharm. 88 (1920), 113.

ether, or whether some alkali is added to the solvent. According to Cloetta, pure digitoxin is prepared from the crude product by dissolving the latter in warm alcohol and adding warm water to this solution until the alcohol-content is about 40 per cent. As the solution cools, digitoxin separates in glistening crystals that appear after several recrystallizations as beautiful plates. This plate-form is characteristic of this manner of crystallizing digitoxin. It is always obtained in the same microscopic form when the product is pure.

Pure digitoxin is soluble in alcohol, acetone, chloroform, glacial acetic acid, soluble with great difficulty in ether, and insoluble in water. From solution in alcohol and chloroform it is precipitated by ether in beautiful crystalline plates. Keller's reaction is characteristic of digitoxin. Elementary analysis gives digitoxin the formula $C_{44}H_{70}O_{14}$. A solution of 0.4 mg. of digitoxin in warm dilute alcohol injected into a lively frog weighing about 35 grams will cause stasis of the heart in 12-15 minutes.

Cloetta has given the following brief summary of the results of his exhaustive investigations

Digitoxin heated in high vacuum yields a compound that sublimes and melts at 114° . It is soluble in water, alcohol, ether, chloroform, acetone, glacial acetic acid, and crystallizes very well. The blue-green color given to glacial acetic acid in Keller's test is even more intense than that given by pure digitoxin. This volatile substance gives none of the sugar reactions, adds bromine and iodine, has the elementary formula $C_8H_{14}O_4$, and is pharmacologically inactive. Cloetta designates the residual substance, remaining after this volatile product has separated from digitoxin, as digitan having the formula $C_{36}H_{56}O_{10}$. Its pharmacological behavior is like that of digitoxin. Acids break up digitan into 1 molecule of digitoxigenin and 2 molecules of digitoxose.

The result of acid cleavage of pure digitoxin is the quantitative formation of 1 molecule of digitoxigenin, 2 molecules of digitoxose, and 1 molecule of an oily substance. The latter is the equivalent of the substance that sublimes in crystals. A close relationship exists between the two products. The crystals treated with acid yield a substance apparently identical with the oil. This oil gives the digitoxose reaction strongly, is not a sugar, and is pharmacologically inactive. A characteristic of digitoxigenin, obtained from digitoxin by acid cleavage, is that it first gives a yellow ring in Kel-

ler's reaction and then colors the glacial acetic acid light green. Agitation produces a stable, fluorescent, emerald-green color, whereas that given by impure digitoxigenin is reddish. Pure digitoxigenin melts at 245° and has the elementary formula $C_{24}H_{36}O_4$. It still has a distinct action upon the heart but this differs somewhat qualitatively and quantitatively from that given by digitoxin. Yet at the same time digitoxigenin is a powerful central convulsive poison.

Digitoxigenin warmed with acid yields anhydro-digitoxigenin, $C_{24}H_{34}O_3$, melting at 184° . It may be obtained direct from digitoxin after more intense acid cleavage. These cleavages proceed quantitatively. Anhydro-digitoxigenin, though no longer active, possibly still causes convulsive action.

The action of digitoxin upon the heart is to be ascribed to the presence of several hydroxyl-groups. Digitoxin treated with benzoyl chloride or stearyl chloride yields crystalline substitution products into the formation of which five acid radicals always enter. These derivatives are pharmacologically inert. When only two hydroxyl-groups are closed, the action of the product upon the heart is weakened.

Benzoylation of digitan also reduces the action of this substance upon the heart.

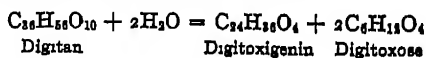
Acetylation of digitoxigenin introduces only one acetyl-group. This acetyl derivative no longer exerts an action upon the heart but possibly still retains convulsive action. In this respect its behavior is similar to that of digitoxigenin. Consequently in both preparations the hydroxyl-group of digitoxigenin responsible for heart action has been put out of commission, whereas the cause of convulsive action apparently must be looked for in some other group.

The cleavages of digitoxin and digitan may be expressed in the following equations:

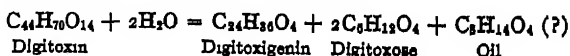
(a) Cleavage of digitoxin *in vacuo*:



(b) Quantitative acid cleavage of digitan:



(c) Primary acid cleavage of digitoxin:



In the last cleavage the elementary formula of the volatile crystals has been arbitrarily introduced for the oil, since it comes nearest to the actual relations.

Benzoyl-digitoxin.—By dropping benzoyl chloride with good agitation into a pyridine solution of digitoxin cooled at 0° , Cloetta obtained pentabenzoyl-digitoxin. Addition of petroleum ether to a benzene solution of this compound causes it to appear in rosette-shaped crystals made up of small rods. It is easily soluble in ether, acetic ether, benzene and chloroform and fairly easily in alcohol. At 145° it sinters and melts at $156-158^{\circ}$.

Keller's Digitoxin Reaction.—Dissolve 0.5-1 mg. of digitoxin in 3-4 cc. of glacial acetic acid containing a trace of iron and carefully add this glacial acetic acid solution to about the same quantity of concentrated sulphuric acid so that the two liquids do not mix. At the zone of contact the blue-green color of the glacial acetic acid characteristic of digitoxin develops upward.

Kiliani's Digitoxin Reaction.—Dissolve a trace of digitoxin in 3-4 cc. of glacial acetic acid containing iron (100 cc. of glacial acetic acid and 1 cc. of 5 per cent ferric sulphate solution). Cautiously add sulphuric acid containing iron (100 cc. of sulphuric acid and 1 cc. of 5 per cent ferric sulphate solution) in about the same quantity as an under layer. A dark zone appears where the two solutions meet, above which after a few minutes a blue band is visible. After some time the entire acetic acid layer becomes deep indigo-blue.

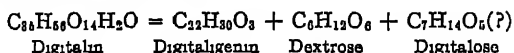
Lafon's¹ Procedure for Digitoxin Test.—In this procedure a well-cooled mixture of equal volumes of 95 per cent alcohol and concentrated sulphuric acid is used together with a very dilute aqueous ferric chloride solution having a scarcely perceptible yellow color. Moisten a small crystal of digitoxin upon a watch-glass with a drop of alcoholic sulphuric acid and then add a drop of ferric chloride solution. An intense blue-green color quickly appears at the line of contact of the two drops. Only digitoxin gives this reaction.

DIGITALIN

Digitalin, $C_{81}H_{136}O_{12}$, occurs only in digitalis seeds (Kiliani). It is somewhat soluble in water (1:1000); easily soluble in alcohol and chloroform; but insoluble in ether. At 210° it sinters and melts at 217° . It tastes faintly bitter and is very active, that is, it is a heart-poison. Boiled in alcoholic solution with very dilute hydro-

¹L. Garnier: Color Reactions of the Toxic Digitalis Glucosides. Journ. Pharm. et Chim. (6) 27 (1908), 369.

chloric acid, it undergoes hydrolysis into digitaligenin and the two sugars dextrose and digitalose:



Concentrated sulphuric acid colors pure digitalin orange-yellow. The color of this solution soon becomes blood-red and upon addition of a little bromine water cherry-red and blue-red. A drop of nitric acid or ferric chloride solution may be substituted for bromine water. This reaction is more certain and of longer duration (1-2 hours), if a trace of digitalin is merely dissolved direct in sulphuric acid, or in Kiliani's sulphuric acid containing iron.

Digitalin dissolved in concentrated hydrochloric acid produces a gold-yellow color becoming upon warming garnet-red to violet-red.

Many recommend the Grandeau and Trapp tests for the detection of digitalin. But these two reactions are not characteristic of digitalin! Consequently they cannot be used for the exclusive detection of digitalin, since with these two reagents many other plant substances give the same or very similar colors. Bing¹ recommends making these tests as follows

1. **Grandeau's Test.**—Add 3 cc. of pure concentrated sulphuric acid to a test portion of substance in a small test-tube and then 3 drops of cold saturated aqueous bromine solution. This mixture when gently shaken will become rose-red to violet-red, if digitalin is present. Among other substances giving a similar color under the same conditions are agaricin, abietic acid, amygdalin, brucine, delphinine, convallamarin, camphor, salicin, solanine, veratrine, cyclamin, cytisine (Bing)

2. **Trapp's Test.**—Pour about 5 cc. of water upon the substance, add an equal volume of phospho-molybdic acid solution and heat the mixture in a boiling water-bath. A green color will appear if digitalin is present. If ammonium chloride is then added to the solution when cold, the green color will change to blue. The solution reheated in the water-bath will become colorless. Under the same conditions the same or similar colors are given by brucine, convallamarin, cyclamin, delphinine, helleborin, heroine, morphine, ricin and strychnine. Aniline hydrochloride and phenacetine also behave similarly. Consequently Trapp's reaction is not characteristic of digitalin!

Detection of Digitoxin in Organs and Urine (Cloetta and Fischer²)

1. **In Organs.**—Render the comminuted material faintly acid with acetic acid, extract with 50 per cent. alcohol, evaporate the

¹ C. Bing. Arch. internat. de pharm. et de thérap. 12 (1906), 337, Pharm. Zentralbl. 45 (1906), 154.

² M. Cloetta and H. F. Fischer. Behavior of Digitoxin in the Organism. Archiv f. experim. Pathol. u. Pharmac. 54 (1906), 294.

filtered alcoholic extract to a syrup, and add alcohol to precipitate salts and dissolved protein substances. Filter, thoroughly extract the precipitate with alcohol, evaporate the extracts to dryness upon the water-bath, dissolve the residue in 10 per cent. alcohol, add a few drops of ammonia, and extract with chloroform. Evaporate the chloroform, dissolve the residue in glacial acetic acid, and use this solution for Keller's or Kiliani's digitoxin test. Or dissolve the residue in 3 cc of chloroform, add 10 cc of ether, 70 cc of petroleum ether, and allow to stand well covered for 48 hours. Decant the solution from the precipitate that forms and examine the latter for digitoxin by the above tests

2. In Urine.—Render the urine faintly alkaline with ammonia and evaporate to a syrup. Stir the residue well with about 5 parts of absolute alcohol and add an equal quantity of chloroform and benzene. After thoroughly shaking the resulting deep brown solution, add basic lead acetate until the supernatant liquid has only a pale yellow color. Filter and wash the precipitate with a mixture of 5 parts of alcohol, 1 part of chloroform and 1 part of benzene. Remove lead from the filtrate with hydrogen sulphide, filter from lead sulphide, and evaporate the filtrate to a thin syrup. Dissolve the latter in 10 per cent alcohol, render alkaline with a little ammonia, and extract repeatedly with chloroform-benzene (3:1). Should these extracts still have a strong color, treat with pure clay (*Bolus alba*). Use the residue from evaporation for Keller's or Kiliani's digitoxin test. About 75 per cent. of 2 mg. of digitoxin added to 500 cc. of urine can be recovered.

Behavior of Digitoxin in the Organism

The heart-mass, according to Cloetta and Fischer (1c), possesses distinctly though in slight degree the power of digitoxin fixation. The quantity of poison adsorbed increases with the weight of tissue used and with the duration of action. Other organs, particularly the liver, manifest the same behavior. Experiments extending over a longer period disclosed the fact that the heart exhibits for digitoxin a distinct attractive power which asserts itself only very slowly. Because of the possibility that heart muscle might have the power of destroying the poison, Cloetta and Fischer studied the behavior of digitoxin as to decomposition by first attempting to destroy it by means of protoplasm and oxidative influences. These experiments showed that digitoxin is capable of resisting isolated tissue as well as the action of hydrogen peroxide, emulsin and invertin. In acute poisonings the poison could also be found again in the animal body. Part of the digitoxin was eliminated though slowly by way of the urine. Digitoxin even manifests a pronounced cumulative action!

Examination of parts of the cadaver for the various principles in digitalis is confined to detection of digitoxin in parts of organs and in urine, since the other digitalis glucosides apparently completely disappear in the organism. Holste¹ studied the action at 37° of different ferments, that is, of diastase, emulsin, pancreatin, trypsin, as well as saliva, gastric and intestinal juice, upon digitalis glucosides, strophanthin, and fresh digitalis infusion. He tested at various intervals of time their activity upon the frog's heart. Following a rather long action of these ferments, digitalis infusion was rendered completely inactive. After shorter action a decrease in activity was observed. Gastric juice and pancreatin had the strongest action. Digitoxin, crystallized digitalin and strophanthin exhibited higher powers of resistance. The uncertainty of digitalis therapy may therefore be attributed, in part at least, to cleavage of the active constituents of digitalis by digestive ferments.

Physiological Detection of Digitalis and Other Substances Having Digitalis Action

Digitalis displays a characteristic paralyzing action upon heart muscle, especially upon the frog's heart, and even in traces may be recognized by this action. For tests upon the entire animal, green frogs are to be preferred on account of their greater sensitiveness to substances having digitalis action. Test animals should be healthy, lively and weigh 30-35 grams. Experiments of this character should be made only by professional pharmacologists or by physicians who have had the necessary training and experience. Four nooses of thick cotton twine are attached to the legs of the experimental frog and then drawn through the slits of the frog-board as shown in Fig. 29.²

By means of curved, pointed scissors cut downward in the region of the arms a small flap of skin. Then grasp with forceps from the side the lower cartilaginous part of the sternum and cut upward a small rectangular opening. Now make a longitudinal incision in the exposed pericardium and press lightly upon the abdomen in proximity to the ventricle. As a result the heart ventricle is pressed out and lies free as shown in the sketch. The opening should be just large enough to expose the ventricle. If it is larger the liver may also be pressed out, especially in case of movement. By means of a hypodermic syringe inject about 1 cc. of an aqueous, alcohol-free solution of the substance in such a manner that about half the solution is quickly introduced into the upper part of one thigh and the remainder into the other thigh. This is done by inserting the fine needle of the syringe, made as sharp as possible, a little below the knee into the lymph-sac in the lower part of the thigh and carefully forcing it from there close under the skin to about the middle of the lymph-sac in the upper part of the thigh. Quickly withdraw the needle after injection and record the time. Then watch for the appearance of systolic stasis of the heart and record the time required. The heart will undergo considerable diminution in size. The duration of the experiment should not be more than 30 minutes. If systole fails to appear during this time, repeat

¹ A. Holste. Behavior of the Substances of the Digitalin Group toward Ferments (Enzymes). *Archiv f. exper. Pathol. u. Pharmacol.* 68 (1912), 323.

From H. Führer. "Detection and Estimation of Poisons Biologically," Urban and Schwarzenberg, 1911, page 71.

the experiment upon a new experimental frog, increasing somewhat the quantity of substance injected

This biological test may be used for the recognition not only of digitoxin but of other "heart poisons," such as strophanthin, convallamarin, scilla, helleborein, oleandrin and adonidin. All these substances exhibit specific "digitalis action."



FIG. 29 —Frog board.

DIGITONIN

Digitonin, $C_{55}H_{90}O_{28}$, is a saponin obtained from the seeds of *Digitalis purpurea*. At most the leaves of the digitalis plant contain only traces. From alcohol it crystallizes in fine needles and in the pure condition is soluble in water with difficulty. When shaken its aqueous solutions foam strongly. It is insoluble in ether and soluble

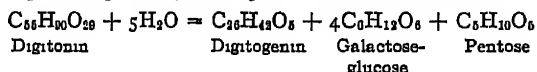
in 50 parts of 50 per cent. alcohol. At 225° it sinters and softens at 235° with yellow color. Pure digitonin gives no color with concentrated hydrochloric acid, nor with the Kiliani-Keller reagent for digitoxin. But a red color is developed with concentrated sulphuric acid and increases in intensity upon addition of a trace of bromine water. Digitonin is not a heart-poison but as a saponin manifests toxic action.

Digitonin-cholesteride has the formula $C_{82}H_{186}O_{30} = (C_{56}H_{90}O_{29} \cdot C_{27}H_{40}O)$. Windaus¹ has furnished proof that saponin substances through addition of cholesterol lose their property of dissolving blood-corpuscles. This cholesterol action moreover is ascribed to formation of well characterized saponin-cholesterides. Digitonin in particular easily gives a beautifully crystallizing cholesteride of the above composition when alcoholic solutions of cholesterol and digitonin are mixed. The precipitate that forms consists of fine needles. But larger crystals of digitonin-cholesteride may be obtained if a little water is added to its solution in boiling methyl alcohol, 100 cc of methyl alcohol at 18° dissolve 0.47 gram of cholesteride. Digitonin-cholesteride is insoluble in cold water, acetone, ether, acetic ether and benzene. The air-dried product contains crystal water which it loses at 110° . In this condition it is very hygroscopic. It has no melting-point but gradually undergoes decomposition when heated above 240° . This cholesteride heated with methyl alcohol is decomposed into its constituents. If it is boiled for 4 hours with this alcohol, then extracted with water and ether, and the ether layer washed with water, pure cholesterol remains when the ether is evaporated. The cholesteride may also be decomposed by hot xylene which takes up cholesterol but not digitonin. Decomposition of 10 grams of digitonin-cholesteride is complete after 10 hours extraction with hot xylene in Stork's apparatus. Digitonin-cholesteride may be used for the quantitative determination of cholesterol and phytosterol. Since cholesterol esters occurring in the animal organism are not precipitated by digitonin, the digitonin method may be used to differentiate and determine quantitatively free cholesterol in presence of cholesterol esters in blood, blood-serum, bile and organs. Phytosterols, plant sterols that also act as antidotes for saponins, give similar complex compounds with digitonin. Digitonin and phytosterol in alcoholic solution form sitosterol-digitoninphytocholesteride, $C_{82}H_{186}O_{30} = (C_{56}H_{90}O_{29} \cdot C_{27}H_{40}O)$. It crystallizes in fine needles and closely resembles digitonin-cholesteride in properties.

Chemistry of Digitonin.—Notwithstanding the ease with which it crystallizes, digitonin can be completely separated only with great difficulty from the other glucosides also present in the seed. This fact explains why crystallized digitonin was not found, until many years after its discovery, to be mixed with about 15 per cent of gintonin. In the light of this fact, it is easy to understand how the work of Kiliani and his students was carried out with a mixture of different

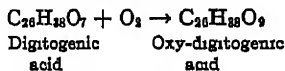
¹ A. Windaus. Cholesterol as an Antidote for Saponins. *Ber. d. Deutsch. chem. Ges.* 42 (1909), 238. Quantitative Estimation of Cholesterol and Cholesterol Esters in Some Normal and Pathological Kidneys. *Zeitschr. f. physiol. Chem.* 65 (1909), 110.

digitalis saponins and how the transformation products described as coming from digitonin were in reality derived only in part from that glucoside and in part from the other glucosides present (Windaus and Weil¹) These two investigators have explained the chemistry of digitonin after careful examination of esters that do not so easily give addition-products, especially by determining methoxyl and by their saponification, and also by employing new methods, that is, by determining according to Zerewitinoff² the number of reactive hydrogen atoms contained in a molecule According to Windaus and Weil, hydrolytic cleavage of digitonin by alcoholic hydrochloric acid into digitogenin, galactose, glucose and a pentose probably takes place as shown in the following equation



Pure, crystallized digitogenin heated with acetic anhydride gives a triacetyl-derivative Upon the basis of their experimental results derived from the latter product, which is also crystalline, Windaus and Weil have assigned to digitogenin the formula $\text{C}_{26}\text{H}_{42}\text{O}_5$ and in part determined its constitution It is a neutral substance containing 3 hydroxyl-groups It contains neither a methoxyl nor a lactone group, nor can aldehyde or ketone groups be detected The two oxygen atoms not present as hydroxyl-groups are probably in an oxide-like union The fact that this substance of high molecular weight can be distilled *in vacuo* without decomposition deserves mention Oxidation of digitogenin by chromic acid gives crystalline digitogenic acid which dried *in vacuo* quite likely has the formula $\text{C}_{26}\text{H}_{38}\text{O}_7 \cdot \frac{1}{2}\text{H}_2\text{O}$ On account of undergoing change at 100° , it cannot be dried and with diazo-methane solution gives a crystalline dimethylester, $\text{C}_{26}\text{H}_{38}\text{O}_7(\text{CH}_3)_2$ By saponifying this and other esters of digitogenic acid with alcoholic potassium hydroxide, digitoic acid, $\text{C}_{26}\text{H}_{38}\text{O}_7$, a stereo-isomer of digitogenic acid is obtained Free digitogenic acid is also a very labile substance and gives, when warmed to 100° or when boiled with dilute caustic alkalis, β -digitogenic acid and digitoic acid As stated above, crude digitonin contains gitonin Digitogenin prepared from it contains gitogenin as an impurity and upon oxidation gives a mixture of digitogenic acid and gitogenic acid According to Windaus and Weil, the three acids, gitonic, desoxy-digitogenic and hydro-digitoic acid, obtained by Kiliani from such an impure digitogenic acid, are identical with gitogenic acid

Careful oxidation of digitogenic acid with potassium permanganate gives tribasic oxy-digitogenic acid, $\text{C}_{26}\text{H}_{38}\text{O}_8$. This acid is also obtained in the same manner and with the same yield from digitoic acid and β -digitoic acid, since 2 atoms of oxygen are required for 1 molecule of acid



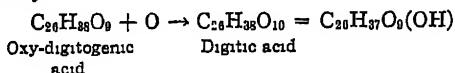
This is proof of the fact that digitogenic acid, digitoic acid and β -digitogenic acid are isomeric.

¹ A. Windaus and K. Weil. Digitonin and its Cleavage-products. *Zeitschr f. physiol Chemie*, Bd. 21 (1922), 62.

² T. Zerewitinoff. Quantitative Estimation of Hydroxyl Groups by Magnesium Organic Compounds. *Ber. d. Deutsch. chem. Ges.* 40 (1907), 2023

The methyl ester, $C_{25}H_{38}O_6(OCH_3)$, of oxy-digitogenic acid is formed by shaking this compound with diazo-methane. The methyl esters of the oxy-digitogenic acids obtained from the three acids, digitogenic, digitonic and β -digitogenic acid, were found to be identical.

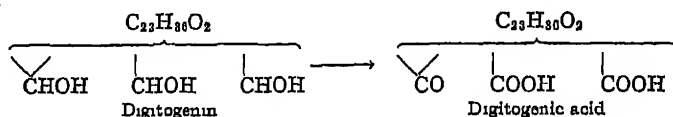
Digitic acid, $C_{26}H_{38}O_{10}$, obtained by oxidizing digitogenic acid, digitonic acid, or oxy-digitogenic acid with strong alkaline potassium permanganate solution (Kiliani) differs from the latter acid in containing one more atom of oxygen present as hydroxyl.



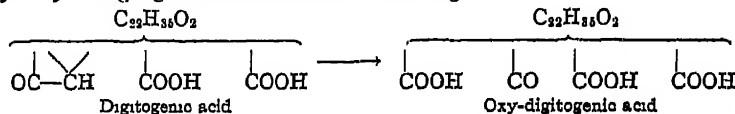
With glacial acetic acid and hydrochloric acid even at 0° , digitic acid loses a molecule each of carbon dioxide and water and passes into "anhydro-digitic acid," $C_{26}H_{36}O_7(?)$. Windaus and Weil draw the following conclusions from the results of their experiments as to the constitution of digitonin and its derivatives.

Digitogenin, $C_{26}H_{42}O_6$, is a saturated triatomic alcohol the two remaining oxygen atoms of which are present in an oxide-like union. Upon the basis of the number of hydrogen atoms present, digitogenin like cholesterol contains four hydrogenized rings.

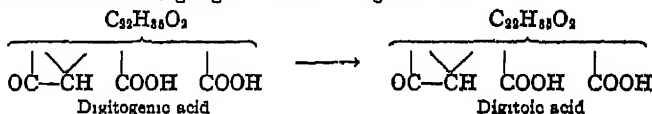
Digitogenic acid, $C_{26}H_{38}O_7$, obtained by the oxidation of digitogenin is a dibasic ketone acid. In all probability a secondary alcohol group is oxidized to the ketone group and at the same time two secondary alcohol groups in the α -position in one ring are converted into two carboxyl groups with breaking of the ring.



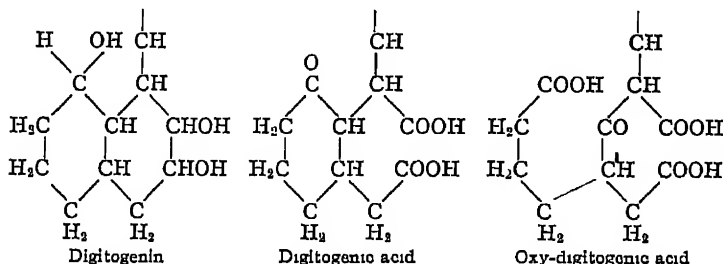
In the conversion of digitogenic acid into tribasic oxy-digitogenic acid, $C_{26}H_{38}O_8$, the group $(CO-CH)$ held in ring-form is apparently oxidized to carboxyl and carbonyl with the opening of a second ring. Consequently the third hydroxyl of digitogenin is also attached to the ring.



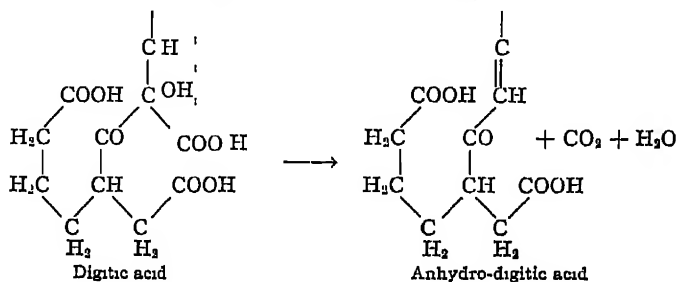
Since the isomeric acids, digitogenic acid and digitonic acid, give the same oxy-digitogenic acid, the supposition is probable that they differ only in the spatial arrangement of the hydrogen atoms in the radical $-\text{CH}$, corresponding to the conversion of digitogenic acid into digitonic acid.



Oxy-digitogenic acid differs from digitoic acid in losing carbon dioxide rather easily, a characteristic both of β -ketone and malonic acids. In this way it was shown that the newly formed ketone or carboxyl group of oxy-digitogenic acid stands in the β -position to one of the carboxyls present, and further that the three hydroxyls of digitogenin are attached to two rings joined together as represented in the following formulae



Digitic acid, $C_{22}H_{38}O_{11}$, an oxy-keto-tricarboxylic acid containing a tertiary hydroxyl, is formed by further oxidation of oxy-digitogenic acid. It loses a molecule of water very easily and then even at 0° a molecule of carbon dioxide, passing into anhydro-digitic acid, a mono-keto-dicarboxylic acid.



STROPHANTHIN

The seeds of various species of *Strophanthus* indigenous to West Africa contain a substance having a bitter taste, a strophanthin. These substances collectively exhibit a typical action upon the heart but they differ from one another in the intensity of this action. If the practice of Thoms¹ is adopted, the various strophanthins are distinguished by placing before the word strophanthin the small initial letter of the name of the particular *Strophanthus* from which it comes. In accordance with this plan the strophanthin from *Strophanthus gratus* is designated g-strophanthin, that from *Strophanthus hispidus*, Kombé and emini, h-, k- and e-strophanthin. Chemically and physiologically these strophanthins of different origin are not identical.

To isolate the "strophanthins," extract with alcohol finely ground *Strophanthus* seeds that have first been extracted with petroleum ether or carbon

¹ H. Thoms: The Strophanthin Question from the Chemical Standpoint. Ber. d. Deutsch. pharmaz. Ges. 14 (1904), 104.

color upon addition of water changes to green with separation of greenish white flocks. Heated with dilute hydrochloric or sulphuric acid, it undergoes hydrolytic cleavage into g-strophanthidin and rhamnose.

Detection of g-Strophanthin

If a solution of g-strophanthin, about 0.01 gram in 1 cc. of water, is carefully added as an upper layer to concentrated sulphuric acid, the latter becomes rose to red and the water layer dirty green. Practically nothing is known with regard to the presence of strophanthin in the animal body and its elimination, neither is it known whether this substance can still be detected in the cadaver following poisoning by strophanthin. As in the case of the digitalis glucosides, the physiological test is the best method of recognizing strophanthin.

Strophanthidin.—Air-dried strophanthidin probably has the formula $C_{27}H_{43}O_7 \cdot 2\frac{1}{2}H_2O$. Over ignited sand $\frac{1}{2}$ molecule of water can be removed. One and two molecules of water can be successively expelled in desiccator over concentrated sulphuric acid, or at 110–125°. It persistently retains crystal water even after recrystallization from methyl or ethyl alcohol. Like strophanthin, crystals of strophanthidin dried as above eagerly absorb water from air without deliquescing. It melts at 169–170°, foams at 176°, solidifies on cooling, and then does not melt until 232°. Strophanthidin is easily soluble in alcohol, acetone, and glacial acetic acid, difficultly soluble in ether, chloroform and benzene, and insoluble in ligroin. It crystallizes from methyl alcohol in beautiful, shining prisms that are frequently united in the form of radiating crystalline warts. $[\alpha]_D^{25} = +45.45^\circ$. Strophanthidin is a neutral substance insoluble in water and sodium carbonate solution. On the contrary, it is soluble warm in caustic alkalies and in barium hydroxide solution. In this respect it behaves like a di-lactone. Strophanthidin dissolves in concentrated sulphuric acid with brick-red color.

Colorimetric Estimation of Strophanthin

(Autenrieth and Quantmeyer¹)

Strophanthin belongs to the class of saponins that act like digitalis (see page 437). Inasmuch as no very satisfactory chemical method of estimating strophanthin is known, pharmacologists use the biological method almost exclusively. The usual custom is to observe the time that elapses from the moment the given solution is injected into the two lymph-sacs in the upper thighs of a frog until the heart is brought to systolic stasis. In this method, as worked out chiefly by Focke for the estimation of "heart poisons," time is the principal factor, whereas Gottlieb determines the smallest quantity that will produce systolic heart-stasis in a large number of experimental frogs within 30 to 45 minutes at most. The best of the chemical methods of estimating strophanthin should be that of Fromme who subjects this substance to hydrolytic cleavage by acid and weighs strophanthidin obtained. The objection to this method is the impossibility of making an accurate quantitative separation of strophanthidin from the large amount of fat present in *Strophanthus* seeds. The color reaction, however, given by official *Kombé-strophanthidin* with concentrated sulphuric acid is well suited for colorimetric determination. Acetic anhydride is a suitable anhydrous solvent to use for strophanthin. Over a concentration range of 0.07–0.40 mg

¹ Not as yet published by the authors.

of strophanthin per cc of solution the tinctorial strength is exactly proportional to the quantity of strophanthin

Requirements.—1 Purest acetic anhydride having a boiling-point of 136.4° . This reagent will remain unchanged for a long time, if kept tightly closed in dark glass-stoppered bottles 2. Purest concentrated sulphuric acid 3 A 5 or 10 cc glass-stoppered graduated cylinder 4 Purest Kombé-strophanthin (Merck) for calibration of the comparison-wedge

Calibration of Comparison-Wedge and Construction of Calibration Curve

Fill the comparison-wedge with a permanent, artificial solution prepared from chrome alum This solution has a green color identical with that of a solution of strophanthin in sulphuric acid For calibrating the wedge use a colorless solution of 1 mg of strophanthin (4) in 1 cc of acetic anhydride With a capillary pipette accurately measure different amounts of strophanthin solution into the small glass-stoppered graduate (3), cool and drop in 1 cc of concentrated sulphuric acid, bring the volume to 5 cc with acetic anhydride and shake After 5–10 minutes the intensity of color has reached a maximum Then introduce a portion of this solution into the glass trough of the colorimeter, and determine the point where the color strength is the same as that of the comparison-wedge, and each time note

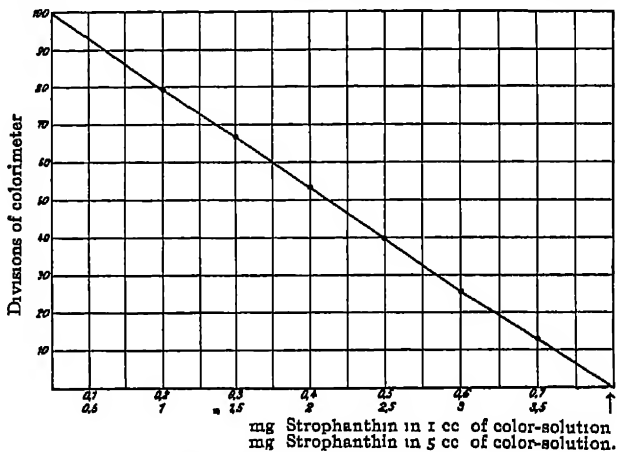


FIG 30—Strophanthin curve

the reading on the scale The mean of these readings is taken as the final result. The shade and strength of the green solutions remain unchanged for about 30 minutes and then gradually pass into olive-green, dirty brown and gold-brown. The following results were obtained in the calibration of a comparison-wedge:

Strophanthin solution in cc.	1	1.5	2	2.5	3	3.5
Strophanthin (mg) per 5 cc of color solution	..	1	1.5	2	2.5	3	3.5		
Strophanthin (mg) per 1 cc of color solution.	0.2	0.3	0.4	0.5	0.6	0.7			
Scale reading for equal color strength		80	67	53	39	25	13		

If the quantities of strophanthin in mg are recorded upon the abscissae of a coördinate system and the readings for equal color strength upon the ordinates, the above curve for the comparison-wedge may be constructed

This comparison-wedge therefore has a measuring range from 0.15-0.75 mg of strophanthin per cc of color solution. In case the color solutions appear too dark, dilute them with acetic anhydride and in the calculation take into consideration the degree of dilution.

Estimation of Strophanthin in Tincture of Strophanthus

In a small porcelain evaporating dish evaporate 2 cc of tincture to dryness upon the water-bath. Stir the residue in the dish while still warm with 5 cc of acetic anhydride and pour the solution through a dry filter. Strophanthin easily passes into solution but other coloring matters in the tincture remain undissolved. Introduce 1, 1.5 or 2 cc. of this acetic anhydride solution into a small glass-stoppered graduate (3), dilute with acetic anhydride to 4 cc, cool and drop in 1 cc of pure concentrated sulphuric acid. After 5-15 minutes, when the intensity of color has reached a maximum, introduce some of the solution into the small glass trough and note the scale-reading at the point where its color strength is the same as that of the calibrated comparison-wedge. Finally ascertain the corresponding strophanthin value on the calibration curve of the comparison-wedge.

Example.—Tincture taken = 2 cc. Solution of the residue from evaporation in acetic anhydride = 5 cc. Used for the colorimetric determination 1 cc = 0.4 cc of tincture. Scale reading for equal color strength is 67 = 0.29 mg in 1 cc, or 1.45 mg of strophanthin in 5 cc of color solution or in 0.4 cc of tincture. So 100 grams of tincture of strophanthus contain $250 \times 1.45 = 362.5$ mg or 0.3625 gram of strophanthin. The quantity of strophanthin found in samples of tincture of strophanthus taken from different apothecaries in Freiburg ranged from 0.32 to 0.39 per cent.

To estimate strophanthin in *Strophanthus* seed, according to directions given in the German Pharmacopoeia, IV Edition, official tincture of strophanthus is prepared by extracting the seeds powdered mediumly fine with 10 parts of diluted alcohol (sp. gr. 0.82-0.86 = 69-68 volumes, or 61-60 parts by weight of alcohol in 100 parts) and strophanthin in this tincture is then determined colorimetrically by the method described above. Preliminary removal of fat from the seeds is not permitted since some strophanthin may be lost in this way. The mean of several determinations of strophanthin in *Strophanthus* seed by this method was 3.15 per cent, whereas the same seed, after removal of fat with ether or petroleum ether, or by direct extraction with water, gave a yield of only 2.16 per cent.

TOAD POISON

By extracting skins of toads with alcohol, Wieland and Weil¹ obtained in conjunction with suberic acid, cholesterol and fatty acids (solid and liquid) a poisonous substance, bufotalin ($C_{36}H_{58}O_6$), appearing in colorless crystals and having an action like that of digitalis. The yield of crude bufotalin before crystallization averaged 20 grams from 2000 toads. According to the latest results obtained by Wieland and Alles,² the nitrogenous substance called bufotoxin, hav-

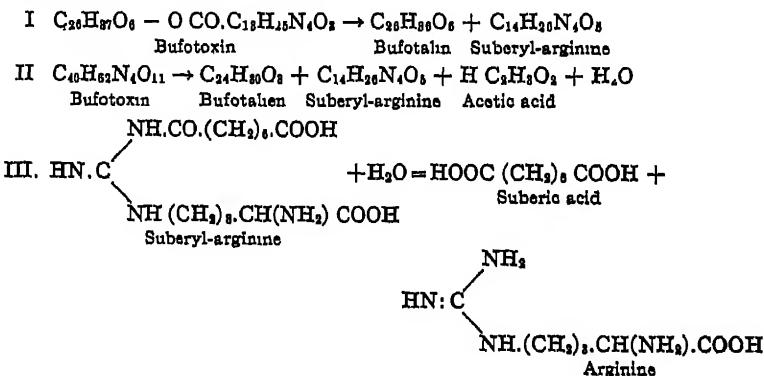
¹ H. Wieland and F. J. Weil. Poison from Toads. Ber. d. Deutsch. chem. Ges. 46 (1913), 3315.

² H. Wieland and R. Alles. The Toxic Substance from Toads. Ber. d. Deutsch. chem. Ges. 55 (1922), 1789.

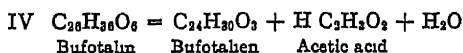
ing the formula $C_{40}H_{82}N_4O_{11}$, is the primary toxic substance from our native toads and was isolated from the fresh secretion of the cutaneous glands. This secretion may be obtained by squeezing the cutaneous glands, that is, the so-called parotis of living toads with forceps having a dull edge. The nearly white milky juice procured in this way from 380 toads, when dried in a vacuum desiccator, gave 4 grams of a hard vitreous mass. Bufotoxin recrystallized several times from alcohol forms colorless druses made up of fine needles melting at $204-205^\circ$. Fusion of this compound is attended with vigorous decomposition. It is practically insoluble in water, ether, acetone, chloroform, acetic ether, and petroleum ether, difficultly soluble in absolute alcohol, more easily soluble in 50 per cent alcohol; and very readily soluble in methyl alcohol and pyridine. Pure bufotoxin is not decomposed after 80 hours boiling in alcoholic solution. It is insoluble in dilute mineral acids, thus exhibiting no basic properties.

Color Reactions (Cholesterol Test).—Dissolve bufotoxin in about 2 cc. of chloroform. Add first a few drops of acetic anhydride and then concentrated sulphuric acid drop by drop. Temporarily the mixture has a red color which changes to blue and finally becomes a beautiful green. Minute quantities of toad poison may be detected by this test. Bufotoxin and bufotalin both give this test but in the case of bufotoxin the first cherry-red phase is even more fugitive than with bufotalin. Bufotalin dissolves in concentrated sulphuric acid with an orange-red color that changes on standing to deep red and exhibits distinct green fluorescence. Cholic acid behaves very much in the same way.

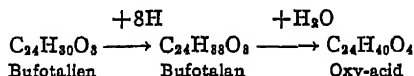
Constitution.—Bufotalin, obtained by Wieland in his earlier experiments with extracts from toad skins, might have come into existence as a kind of "genin" from the bufotoxin primarily present, since the latter is exceedingly sensitive to acids. When boiled for 4-5 hours with dilute alcoholic hydrochloric acid, it readily undergoes hydrolytic cleavage into bufotalen, $C_{24}H_{40}O_8$, and suberyl-arginine, $C_{14}H_{26}N_4O_8$. The bufotalen that separates crystallizes from alcohol in light yellow leaflets melting at 219° . Suberyl-arginine, isolated as such with difficulty, can be decomposed when boiled with strong hydrochloric acid into suberic acid, melting at $139-140^\circ$, and arginine. The latter was recognized by its nitrate and picrolonate (mpt 230°). According to Wieland and Alles, conversion of bufotoxin into bufotalin and the hydrolytic cleavage of the former may be expressed by the following equations



According to Wieland, bufotalin, $C_{28}H_{38}O_8$, the nucleus of bufotoxin, is a derivative of a molecule containing 24 carbon atoms which like the bile-acids may be composed of four hydro-aromatic rings. The remaining two carbon atoms in bufotalin make up an acetyl-group present in the form of an ester. This group may be removed by hydrolysis as acetic acid. Bufotalin is also a lactone and contains two hydroxyl-groups, one secondary and the other tertiary. Since bufotalin adds four atoms of hydrogen when subjected to catalytic hydrogenization and is converted into the saturated hydro-bufotalin, $C_{28}H_{40}O_8$, two ethylene linkings must be present in the molecule. When treated with concentrated hydrochloric acid, bufotalin loses a molecule each of acetic acid and water, becoming bufotalien, $C_{24}H_{30}O_8$, which has a yellow color and contains four ethylene linkings.



Bufotalien is the bridge to the bile-acids, since hydrogenization (+ 8 H-atoms) converts it into the saturated bufotalan, $C_{24}H_{38}O_8$, which still contains the lactone-group. The oxy-acid, $C_{24}H_{40}O_4$, corresponding to this lactone, is isomeric with desoxy-cholic acid obtained from the bile-acids.



Desoxy-cholic acid may be regarded as a dioxy-cholanic acid. Cholanic acid, $C_{24}H_{40}O_2$, is probably present as a common basal substance in bufotalin, the first cleavage-product of bufotoxin from toad poison, as well as in the bile-acids and cholesterol. Like cholesterol the bufotoxin molecule contains one ethylene linking.

Physiological Action.—Both bufotoxin and bufotalin exhibit pronounced digitalis action, bringing the frog's heart to systolic stasis and also having strong haemolytic action. In this respect the two toad poisons behave like saponins.

SAPONINS OR SAPONIN SUBSTANCES

The term saponins, or saponin substances, includes a large number of glucoside-like substances of widespread occurrence in the plant kingdom having in common certain chemical, physical, and especially physiological properties. When shaken, their aqueous solutions readily foam. In this respect they resemble soaps. Many saponin substances have a sharp, harsh taste. In powdered form they excite violent sneezing. They are capable of holding many finely divided substances in a state of emulsion. Saponin substances dialyze slowly and very incompletely. They may be partially salted out from their solutions. Excepting the gluco-alkaloid solanine, which contains nitrogen and is alkaline, saponins may be classified chemically as nitrogen-free glucosides.

At the present time, chemical knowledge of saponins and of their hydrolytic cleavage-products is still very defective, because of the extreme difficulty involved in getting them pure and establishing their exact identity on account of their amorphous character.

With few exceptions saponins dissolve easily in water and their solutions foam strongly. They are also colloidal and possess an emulsifying action. Saponins dissolve with tolerable ease in hot dilute alcohol and in methyl alcohol, only very slightly in absolute alcohol and acetone, and not at all in ether and petroleum ether. Saponins dissolve with relative ease in phenol and less easily in isobutyl and isoamyl alcohol. Some saponin solutions are optically active others inactive. The application of drugs containing saponins in washing, in preventing finely divided substances from settling, and crystalline substances from crystallizing depends upon the emulsifying properties of saponin-substances. Some saponins are feebly acid in character. These are the saponin-acids. Other saponins react neutral and are chemically indifferent. These are the saponotoxins. Frequently saponin-acids and saponotoxins occur together in one and the same plant. Consequently they must be related to one another. Reagents used to precipitate saponins from concentrated aqueous solution are barium hydroxide in saturated aqueous solution, neutral and basic acetate of lead, and ammonium sulphate.

Like soaps and many proteins, saponins may be salted from aqueous solution by ammonium sulphate and various other soluble salts. Saponins to a great extent are separated from solution by animal charcoal and by lead sulphide as a result of adsorption. Boiling alcohol will redissolve what has been adsorbed. Saponins also exert a solvent action upon red blood-corpuscles. They destroy the stroma of the corpuscles and haemolysis takes place. Saponins withdraw lecithin, and partially also cholesterol, forming with them labile molecular compounds and bringing the blood-corpuscles into solution. (See "Physiological Action of Saponins.")

Hydrolytic cleavage of saponins by dilute acids (3-4 per cent sulphuric acid), which frequently runs to completion very slowly, usually gives amorphous compounds called sapogenins. At the same time certain sugars, such as glucose, galactose and pentoses, are formed. Some sapogenins are crystalline but in general they are even more difficult to purify than saponins, for their composition seems to depend upon external experimental conditions.

Occurrence of Saponins.—Evidence of the wide distribution of saponins in the plant kingdom is afforded by the fact that up to the present time more than 200 saponin-substances have been found in over 50 plant families including mono- and dicotyledonous plant species. The parts of plants containing saponins are roots (Senega, Saponaria), tubers (Cyclamen), barks (Quillaja, Guaiacum), fruits (Sapindus, Saponaria), seeds (Aesculus, Agrostemma, Thea), stems (Dulcamara), and leaves (Guaiacum). In fact there is scarcely any part of the plant organism in which saponins may not occur. The plant families producing saponin substances more abundantly are the caryophyllaceae, colchicaceae, polygalaceae, sapindaceae and sileneae. Quite considerable quantities of saponins may occur in the particular part of the plant. In the following some of the exhaustively investigated saponins, differentiated according to origin, will be mentioned.

Saponin from Soapwort.—*Saponaria officinalis* contains 4-5 per cent. of saponin. E. Schmidt isolates it by extracting finely ground soapwort with 90-91 per cent alcohol, collecting the precipitate that separates from the hot filtered extracts after 24 hours, washing with ether and drying. This precipitate is

purified by dissolving in a little water and reprecipitating with barium hydroxide. Precipitated barium hydroxide saponin is washed with barium hydroxide solution and decomposed with carbon dioxide. The filtered aqueous solution is evaporated and saponin precipitated by alcohol and ether. This saponin is a white, amorphous powder dissolving readily in water, slightly in alcohol and insoluble in ether.

Quillaja-Saponins.—The bark of *Quillaja saponaria* contains as high as 8.8 per cent of saponins. Quillajic acid, which Kobert designates as the physiologically active toxic modification of Quillaja-saponins, is characterized by a strong and persistently harsh taste. Neutral lead acetate precipitates it from aqueous extracts of Quillaja bark. Basic lead acetate added to the filtrate from this precipitate causes further precipitation of a very poisonous compound, Quillaja sapotoxin! One and the same sapogenin has been obtained in crystalline form from quillajic acid and Quillaja-sapotoxin.

Saponins of Senega Root.—Polygalic acid and senegin, showing great similarity in behavior to quillajic acid and the sapotoxin, have been isolated from the root of *Polygala senega*.

Guaiaac-Saponins.—The wood and bark of *Guaiaacum officinale* contain two saponins, namely, guaiaac-saponic acid precipitated by lead acetate and guaiaac-saponin precipitated only by basic lead acetate. Both saponins are characterized by slight haemolytic properties and consequently are not very toxic.

Sapindus-Saponin.—This substance was isolated from the fruit of various species of *Sapindus*, for example, *Sapindus Rarak* DC (May¹). The shells on the average contain 13.5 per cent of saponin. It is a white, amorphous powder and very readily soluble in water. Barium hydroxide and basic lead acetate precipitate it but not quantitatively. It has a powerful haemolytic action. This saponin is hydrolyzed when warmed with 5 per cent hydrochloric or sulphuric acid into a sapogenin, $C_{12}H_{22}O_8$, and one molecule each of a hexose and a pentose.

Winterstein and Maxim have shown that a naphthalene ring is the basis of the sapogenin from *Sapindus* as well as of that from the horse-chestnut, for these substances upon oxidation with nitric acid yielded a dinitro-naphthalene together with oxalic acid.

Saponin of Corncockle, *Agrostemma*-Sapotoxin.—Remove oil from coarsely powdered seeds of *Agrostemma githago* by means of ether and then extract for 2-3 days with 70 per cent alcohol (5 liters for 2500 grams of seed). Evaporate the alcoholic extract upon the water-bath to the consistency of thick syrup and then stir with absolute alcohol. At first the residue passes into solution but further addition of alcohol precipitates the sapotoxin which soon becomes solid and pulverulent. Corncockle seed contains about 6.44 per cent of fat. The yield of crude sapotoxin is 4.9-6.1 per cent, or at most 7.7 per cent. (Brandl²). Pure sapotoxin was obtained by a method of purification with lead acetate and basic lead acetate and extraction with alcohol. It is a light yellow, water-soluble

¹ O. May. Chemico-Pharmacological Investigation of the Fruit of *Sapindus Rarak*. D. C. Archiv d. Pharmaz. 244 (1906), 251.

² J. Brandl. Sapotoxin and Sapogenin from *Agrostemma Githago*. Archiv. f. experim. Pathol. u. Pharmacol. 54 (1906), 245 and 59 (1908), 245.

powder giving no turbidity with lead acetate, a greenish white precipitate with basic lead acetate, and a yellowish precipitate with barium hydroxide solution. Concentrated sulphuric acid dissolves this sapotoxin with yellow color gradually changing to purplish red and exhibiting an absorption-band in the spectrum at E. The boiling solution reduces ammoniacal silver nitrate solution but has no action upon Fehling's solution! Three hours boiling with 4 per cent sulphuric acid hydrolyzes it into a crystalline sapogenin and various sugars. Diluted 1:50,000, *Agrostemma*-sapotoxin causes haemolysis in a short time. Treated with cholesterol, *Agrostemma*-sapotoxin even to the extent of 0.01 gram fails to produce haemolysis. Injection of 12-15 mg of this sapotoxin into the lymph-sac in the back of a frog paralyzes the animal within 3 hours.

Using Kobert's method of purifying saponins, Brandl isolated a new substance called agrostemmic acid from the precipitate caused by neutral lead acetate. This product constituted 6-7 per cent of the crude sapotoxin. *Agrostemmic* acid is a brownish white powder differing from *Agrostemma*-sapotoxin principally in its behavior with lead acetate with which it gives a precipitate. This acid produces the same poisonous effects caused by the sapotoxin.

General Reactions and Detection of Saponins

1. **Foam Test.**—Aqueous saponin solutions when shaken foam more or less strongly as soap solutions do.

2. **Sulphuric Acid Test.**—Concentrated sulphuric acid dissolves saponins with yellow color gradually changing to red, purplish red, and often red-violet or blue-green.

3. **Froehde's Test.**—With various saponins Froehde's reagent produces a blue-violet and frequently also a green color.

4. **Brunner-Pettenkofer Bile Acid Test.**—Since saponins are glucosides they give this test. According to Gadamer, the substance to be tested should be dissolved in a little water with a particle of purified ox-gall (*Fel Tauri depuratum*). This solution should then be added as an upper layer to an equal volume of concentrated sulphuric acid in a small test-tube. If a saponin is present, a blood-red zone will appear at the contact-surface of the two liquids and the entire mixture will become red when shaken.

Since nearly all sugars give the Brunner-Pettenkofer test, a positive result indicates a saponin only in absence of sugar. Glucosides, and consequently saponins, do not reduce Fehling's solution in the cold. Many sugars, however, that come into question do, and so it is frequently possible to decide by means of Fehling's test whether sugar or glucoside is present. In consequence of the cleavage of sugar as a result of hydrolysis by dilute acids, saponins have a reducing action upon Fehling's solution. In this respect cane-sugar behaves like a saponin. To detect saponin positively in presence of cane-sugar, the former should be extracted with an appropriate solvent or first precipitated with neutral or basic lead acetate and then set free from the precipitate with hydrogen sulphide.

5. **Haemolytic Test.**—If a solution of a saponin in 0.9 per cent. sodium chloride solution is added to 1 cc. of a mixture of 1 cc. of defibrinated blood and 100 cc. of 0.9 per cent sodium chloride solution, red blood-corpuscles gradually dissolve and for this reason the originally turbid mixture becomes clear and laked.

6. **Fish Test.**—Most saponins exert a strong, specifically toxic action upon fish. Dilute with ordinary fresh water the solution to be tested for saponin and place in it a small fish. After 12–24 hours, if a saponin is present, the fish will become torpid and finally die.

Physiological Action of Saponins

Most saponin substances possess toxic properties. If introduced directly into the blood, their poisonous action is far greater than if taken internally by the mouth. Since saponins are usually absorbed with difficulty, persons in good health can tolerate them in large quantities taken internally in dilute solution by the mouth without deleterious effects. Toxic saponins manifest in common the property of irritating protoplasm which in larger doses they can kill. In various ways they prove that they are protoplasmic poisons. The action of saponins upon blood-corpuscles is also in harmony with this behavior. In fact R. Kobert and his collaborators have shown defibrinated blood diluted 100 times with physiological salt solution to be the best and most convenient reagent for saponin substances. Saponins cause haemolysis and the blood solution becomes laky. Agglutination and formation of methaemoglobin do not take place. The freer the blood of serum, the more pronounced is the haemolytic action of saponin substances upon blood-corpuscles. Recent investigations have shown that saponins act more vigorously upon blood-corpuscles isolated from serum because blood-serum contains cholesterol which exerts a protective influence and retards haemolysis. Quite likely haemolytic action of saponins is due to removal of cell membrane lecithin, the chief constituent of the cell-wall, from red blood-corpuscles, for lecithin-saponins are formed. Saponins also combine with cholesterol, as well as with lecithin, forming cholesterol-saponins. Once the affinities of a saponin have been satisfied by cholesterol, it ceases to act upon the lecithin of the membrane of blood-corpuscles. Consequently cholesterol prevents a saponin from causing haemolysis and so acts as an antidote for saponin substances. Ransom¹ has made the important discovery that addition of cholesterol checks the solvent action of a saponin upon blood-corpuscles. At first it was not known whether this antidotal action was due to a chemical reaction, or to adsorption, that is, to a physical process. R. Kobert,² as well as Madsen and Noguchi,³ was able to dissolve cholesterol, which is insoluble in water, in an aqueous saponin solution. They assumed that this physiologically inactive solution contained a labile saponin-cholesterol compound no longer having haemolytic power. Recently Windaus⁴ has definitely proved that saponin-cholesterides exist. Digitonin-cholesteride, $C_{56}H_{102}O_{29}$, $C_{27}H_{46}O$, crystallizes in fine needles, when a hot alcoholic solution of digitonin (1 molecule) is poured into a similar solution of cholesterol (1 molecule). This cholesteride is

¹ F. Ransom: Saponin and its Antidote. *Deutsche med. Wochenschr.* 1901, page 194.

² R. Kobert: *The Saponins*. Stuttgart 1904.

³ T. Madsen and H. Noguchi. *Toxins and Antitoxins, Saponin, Cholesterol*. *Chem. Zentralbl.* 1905 I. 1265

⁴ A. Windaus: A Saponin-Cholesterol Compound, *Archiv. f. exper. Pathol. u. Pharmacol.* 64 (1911), 141.

formed without elimination of water. Hence in this reaction between digitonin and cholesterol we are dealing most probably with the formation of a molecular compound. Then later Yagi¹ obtained from the easily crystallizable and strongly haemolytic dioscin from *Dioscorea Tokoro* a crystalline cholesterolide. This dioscin-cholesteride has the composition $3C_{24}H_{38}O_9 \cdot 2C_{27}H_{46}O \cdot H_2O$ (or $2H_2O$) and is formed by pouring together equi-molecular alcoholic solutions of the components. First a jelly separates and then changes in 12 hours into a crystalline mass. From alcohol it forms a colorless, micro crystalline powder melting at 223° , very slightly soluble in water, slightly soluble in alcohol, methyl alcohol, ether and chloroform, but tolerably easily soluble in boiling alcohol and methyl alcohol. The alcoholic solution diluted with much water does not foam. The cholesterolide is without haemolytic action.

Saponin solutions also dissolve white blood-corpuscles but only at higher concentrations. A physiological action characteristic of many saponins is exhibited in the stupefaction and killing of fish, even in water containing only 1:200,000 of saponin substance (R. Kobert).

Saponin substances administered internally by the mouth manifest relatively slight toxic action, at least upon dogs (Wacker²). Saponin, administered by the mouth daily for 6 weeks to dogs weighing about 7 kilograms, was well tolerated in doses of 0.5 gram, whereas larger quantities caused vomiting. It was tolerated better with meat than when dissolved in water or milk. Examination of the dead animal disclosed deposits of fat in the tissue of the kidneys.

In this connection mention should be made of experiments by Brandl³ who fed various animals with *Agrostemma-sapotoxin* and corncockle meal prepared by him. Rabbits tolerated large quantities of these substances taken by the mouth. On the other hand, swine and dogs reacted tolerably easily but gradually became accustomed to quite considerable quantities of *Agrostemma-sapotoxin*. Upon the whole, relatively large quantities could be taken without injury. Consequently in animals poisoned by corncockle, disease changes of the digestive tract must have already existed. In that case a local poison having a strongly irritant action need not be introduced in large quantity to cause acute poisoning. Under such conditions small quantities of corncockle may exercise an unfavorable influence upon the digestive organs.

Rendering Saponins Non-toxic.—Saponins may be rendered non-toxic by cholesterol (see above), by treatment with hot barium hydroxide solution, and by saponification of the acetyl derivative with barium hydroxide solution or alkalis. Haemolysis serves to distinguish a toxic saponin from one that has been rendered non-toxic, since only toxic saponins act haemolytically. Saponins that have been rendered non-toxic and also glycyrrhizin cause no haemolysis whatever. The same is true of the saponin-free residues obtained by the Brunner-Rühle method of extraction (Halberkann).

¹ S. Yagi. A Saponin-Cholesterol Compound. *Archiv. f. exper. Pathol. u. Pharmacol.* 64 (1911), 141.

² L. Wacker. Action of Saponin Substances. *Biochemische Zeitschr.* 12 (1908), 8.

³ J. Brandl. Toxicity of Corn-cockle Seeds. *Landwirtsch. Versuchsstation* 72 (1910), 326.

Variation in Strength of Haemolytic Action.—Because of differences they manifest in haemolysis, saponins introduced into the circulation exhibit different degrees of toxicity, although it is not possible to trace their toxic action exclusively to haemolysis. Then like blood-corpuscles all other cells of the organism, for example, the ganglion-cells of the brain, are also attacked by saponins. These cells require smaller doses than blood-corpuscles do and action upon them takes place more slowly. Intravenous injection of these small doses, which are quite insufficient for haemolysis, may cause death in 4–6 days. Use of plants containing saponin in fishing depends upon this action. The fish breathe through their gills and take the saponin dissolved in water direct into the circulation by which they are stupefied or killed, even in a dilution of 1 : 240,000.

Detection of Saponins in Effervescing Beverages

(Brunner-Rühle¹)

If acid, neutralize 100 cc or more of the given beverage with magnesium carbonate and evaporate upon the water-bath to 100 cc. Then shake this solution vigorously with 20 grams of ammonium sulphate and 9 cc of liquid phenol. The phenol layer containing the saponin, after separation from the aqueous layer, is shaken with 50 cc of water, 100 cc of ether and, if necessary, with 4 cc of alcohol to prevent formation of emulsion. Separation of the two layers may be facilitated by gently warming the contents of the separatory funnel. The aqueous layer containing the saponin is drawn off, evaporated upon the water-bath at about 60°, and the residue dried in a vacuum desiccator. The dry residue is further purified by pouring over it 1–2 times 10 cc portions of acetone and each time allowing it to stand for 24 hours. Acetone does not dissolve saponin and is poured off. The part of the residue remaining undissolved is tested for saponin. To detect saponin, dissolve this residue in warm water. This solution will foam strongly when shaken if saponin is present. Divide the solution into four equal portions. Evaporate one portion upon the water-bath with gentle heat and test the cold residue with concentrated sulphuric acid. Also evaporate the second portion, dilute the aqueous solution of the residue with ordinary water and introduce a small, lively fish. If a saponin is present, stupefaction and death of the fish will take place after 12–24 hours. The third portion after evaporation is taken up in 0.9 per cent sodium chloride solution and used for the haemolytic test. If haemolysis is detected, evaporate the fourth portion and take up the residue in 0.9 per cent sodium chloride solution. Shake this solution with an ether solution of cholesterol, taking 1 part of cholesterol to 20 parts of saponin solution and warming the mixture for several hours at 36°. The saponin solution now freed from poison by cholesterol should after evaporation of ether give a negative haemolytic test.

Notes.—According to Rühle, the guaiac-saponins prepared from wood and bark of *Guaiacum officinale* show, contrary to other statements, distinct haemolytic action. Neutral lead acetate precipitated from commercial saponin the acid constituent, saponic acid of Guaiac bark, and basic lead acetate the neutral saponin of Guaiac bark. Both saponins are white, pulverulent products that

¹ J. Rühle. Detection of Saponin. *Zeitschr. f. Unters. von Nahrungs- u. Genussmitteln* 16 (1908), 165

cause coughing and give aqueous solutions that foam strongly. In dilution 1:1000 they cause complete haemolysis and partial haemolysis in dilution 1:5000. The acid saponin has a somewhat stronger haemolytic action than the neutral body.

Coal-tar Dyestuffs.—Examination of effervescent beverages showed that they were colored with coal-tar dyes. These dyes were found in the residues tested for saponin by their haemolytic properties. In testing eight light red, yellow and green dyes, used collectively in the preparation of effervescent beverages, Rühle¹ failed to find either in glass or under the microscope any evidence of haemolysis caused by these dyes.

Dextrin.—Liquids containing dextrin are neutralized with magnesium carbonate, evaporated to 20 cc. and at once treated with 150 cc. of 96 per cent alcohol. The mixture is allowed to settle for 0.5 hour, heated to boiling upon the water-bath, filtered hot, and the filtrate freed from alcohol after addition of a little water. The residue is finally diluted to 100 cc. with water and this solution is shaken vigorously with 20 grams of ammonium sulphate and 9 cc. of liquid phenol. In other respects the procedure is the same as described above. Effervescent lemonade powder and similar preparations containing saponin are dissolved in water to 100 cc., the solutions heated to boiling, filtered when cold and examined according to the Brunner-Rühle method.

Detection of Saponin in Oil-emulsions.²—Dilute 100 grams of the given oil-emulsion in a capacious beaker with the same volume of water. Stir well while adding gradually 400 cc. of 95-96 per cent alcohol, continue stirring during 2 hours and allow to stand for 24 hours. Filter the clear liquid from the underlying fat mass through linen cloth, repeat the extraction of fat with 60-65 per cent alcohol, and again filter. Neutralize the combined filtrates with sodium carbonate and concentrate upon the water-bath to 100 cc. Extract with ether any aromatic substances present and according to the Brunner-Rühle method add 20 grams of ammonium sulphate and shake with 9 cc. of liquid phenol. Repeat this extraction several times. Then shake the combined phenol extracts with a mixture of 100 cc. of ether, 30 cc. of water and 5 cc. of alcohol, and allow the mixture to stand for 24 hours. All saponins will have passed into the aqueous layer which is evaporated upon the water-bath. First extract the residue with acetone and test for saponin according to directions given above.

Detection of Saponin in Beverages

(Müller-Hössly³)

This method of detecting saponin takes advantage of the property these substances have of concentrating in the foam when their solutions are shaken.

Procedure.—Neutralize in a liter cylinder 500 cc. of the beverage (1000 cc. if very little foam is formed), set the cylinder in a large funnel, and pass air through

¹ J. Rühle. Detection of Saponin, III Paper. *Zeitschr. f. Unters. d. Nahrungsmittel* 27 (1924), 192.

² E. Carlinfant and P. Marzocchi. Estimation of Saponin and Saccharin in Oil Emulsions. *Boll. Chim. Farm.* 50 (1911), 609.

³ E. Müller-Hössly. A Simple Test for Saponin. *Mitteilung f. Lebensmitteluntersuchung u. Hygiene* 8 (1917), 113.

the liquid by means of a tube extending to the bottom of the cylinder. The foam formed soon runs over, collects in the funnel, coalesces and drops into a graduate standing under the funnel. Mix 1 cc of the liquefied foam with 1 cc of blood solution (1 cc of defibrinated blood + 99 cc of physiological salt solution) and test for haemolytic action. Continue passing air through the solution until foaming has ceased. The residue in the cylinder should not have haemolytic action, if saponin has been positively detected. The sugar-content of beverages, examined by the method just described, has no influence within quite a wide range (20–200 grams of sugar per liter) upon haemolytic action. Not until the quantity falls below a certain minimal value (20 grams per liter) does haemolytic action appear even in absence of saponins and then solely because the osmotic pressure is too low. In an examination of beer 500 cc should be taken and first neutralized with sodium carbonate until litmus paper shows a distinct blue color. The first 5 cc should be used for the haemolytic test, for in the case of beverages and beer saponin is present in higher concentration.

Notes.—The extraordinarily high concentration of saponin in the foam constitutes the value of this method. If the operation is carried out repeatedly, using rather small containers, it is possible to reach a concentration of over 300 mg of saponin per liter.

Detection of Saponin in Presence of Glycyrrhizin

According to Rühle,¹ the color changes given by concentrated sulphuric acid and Froehde's reagent cannot be relied upon in all cases for the positive detection of saponin, not to speak of its positive differentiation from glycyrrhizin, since residues from aqueous solutions contain many other substances, such as sugar and dyes, that interfere with these reactions. The reactions with α -naphthol and thymol, given by sugar as well, also fail. The same is true of the Brunner-Petenkofer reaction with purified ox-gall which is a general reaction for sugar depending upon the cleavage of sugar from glucosides. Rühle has shown experimentally that by means of haemolysis saponin may be easily and positively detected in presence of considerable quantities of glycyrrhizin and that the latter cannot disguise presence of saponins.

In performing this reaction 0.9 per cent salt solution (= Solution I), also called physiological salt solution, should be used for all solutions and dilutions. The blood solution should be defibrinated blood diluted to 1 per cent. with Solution I (= Solution II), or a suspension of red blood-corpuscles in Solution I corresponding in concentration to Solution II (= Solution III). The latter solution is used when only very little saponin is present, that is, to increase the action in consequence of removal of cholesterol from the serum. Dissolve the residue obtained by evaporating the given aqueous solution (see above) in Solution I for the saponin test. Carry out the reaction in small test-tubes holding about 10 cc and always add for every 1 cc. of Solution II or III successively 1, 2, 3 cc of the solution prepared for the test. Note whether the solution becomes clear. If it does, then add cholesterol to 10 cc of the original solution showing haemolytic action and test again to ascertain whether haemolytic action has now disappeared or

¹ J. Rühle: Detection of Saponin, II Paper. *Zeitschr. f. Unters. der Nahrungsmittel* 23 (1912), 566.

not If it has disappeared, positive proof is furnished that the given material contains saponin In presence of very little saponin, or of substances causing turbidity such as glycyrrhizin, the reaction should be followed under the microscope

Notes.—According to Sorman,¹ certain precautions should be observed in testing for saponin by haemolysis All liquids examined should be isotonic and contain no substances such as acids, alkalies, alcohol and ether that may interfere with the reaction High temperatures should be avoided and liquids should be approximately sterile The suspensate of red blood-corpuscles used for the haemolytic test is prepared by washing defibrinated blood three times with physiological salt solution in a centrifuge When the salt solution remains perfectly clear, 5 cc of the centrifugalized red blood-corpuscles are diluted to 100 cc with physiological salt solution Such a suspensate will keep in an ice-box for 2-3 days

To detect saponin by haemolysis in Seltzer water, beer and urine, neutralize the given liquid, expel carbon dioxide and alcohol, and finally make it isotonic with sodium citrate Then mix such a solution with the above-mentioned suspensate of red blood-corpuscles and keep for several hours at low temperature.

In case of red wine, tannic acid compounds should first be removed by means of albumin or gelatin The delicacy of this test in case of beer was 1:100,000, in case of Seltzer water 1:40,000

Detection of saponin in flour, according to Rusconi,² requires kneading upon a tight cloth 5 grams of flour and 4 grams of 2 per cent neutral sodium citrate solution, adding the citrate solution drop by drop Filter 16 cc of citrate solution obtained and add to 3 cc of clear filtrate 1 cc of the suspensate of red blood-corpuscles After the un-haemolyzed blood-corpuscles have settled, if the supernatant liquid appears red and transparent, saponin is present To detect saponin in bread (detection of *Agrostemma*), heat 10 grams of powdered bread to boiling three times, using each time 5 cc of 95 per cent alcohol, 5 cc. of chloroform and pouring quickly through a dry filter Evaporate the filtrates upon the water-bath, dissolve the residue in physiological salt solution, filtering if necessary Otherwise the procedure is the same as described above

Detection of Saponins

(Rosenthaler³)

Some saponins such as guaiac-saponin and saponin from *Bulnesia Sarmienti* exhibit slight or no haemolytic action at all In such cases the method of Rühle is more or less of a failure It is also possible that regenerated saponins without haemolytic action are commercial products. Rosenthaler does not isolate pure saponins but their water-soluble products of hydrolysis designated as pro-sapogenins

Procedure.—Add to the given liquid sufficient hydrochloric acid to bring the percentage up to about 2.5 per cent. First filter the solution, if necessary, and

¹ C. Sorman. Detection of Saponin in Beverages and Foodstuffs. *Zeitschr f Unters. d Nahrungs-u Genussmittel* 23 (1912), 561

² Rusconi. *Giornale di Farmacologia sperimentale* 1912.

³ L. Rosenthaler. Detection of Saponins. *Zeitschr f Unters d Nahrungs-u. Genussmittel* 25 (1913), 154

then heat until hydrolysis is complete, that is, until the liquid no longer foams when shaken. Allow the solution to cool without filtering and shake while still warm with acetic ether, using 5 cc of this solvent for every 100 cc of liquid. Break up any emulsion that may form by adding a little alcohol. Wash the clear acetic ether solution with a little water, using 5-10 cc each time, until Cl⁻ ion has disappeared. Then evaporate the solution, if it has only a slight color, otherwise discharge the color by means of animal charcoal. Use the residue from the acetic ether solution to make the color test with concentrated sulphuric acid. A positive result given by this test is accepted as conclusive. Another portion of residue is dissolved in sodium carbonate solution and the solution shaken to see whether it still foams. Pro-sapogenin dissolves very slightly in water even at boiling temperature but is soluble in acetic ether, ethyl and methyl alcohol, acetic acid and also slightly soluble in chloroform. It possesses acid properties.

To detect saponin in beer, first heat 100 cc of the latter under a reflux with 100 cc of 95 per cent alcohol until a flocculent precipitate appears. Filter this precipitate and use the filtrate, after distilling off alcohol, for hydrolysis as directed above. Employing this method, Rosenthaler was able in every instance to detect saponin. Presence of glycyrrhizin did not interfere with the detection of saponin.

According to Sagel,¹ detection of saponin by the color reaction with concentrated sulphuric acid may actually be hastened and intensified by addition of acetic anhydride. Rub a small quantity of the given product upon a watch-glass with 2 drops of acetic anhydride and then allow 1 drop of concentrated sulphuric acid to run down from the margin. In presence of saponin, a distinct light red color like that of a dilute fuchsin solution immediately appears.

Detection of Corn-cockle in Flour

This test is due to presence in corn-cockle of the saponin githagin. Heat 500 grams of the flour with 1 liter of 15 per cent alcohol, filter hot, evaporate the filtrate to smaller volume, and separate saponin by addition of absolute alcohol and some ether. After the mixture has stood for 12-24 hours, any precipitate that has formed is collected upon a filter, dried at 100° to coagulate admixed protein substances, then dissolved in a little cold water, and saponin again precipitated from the filtered solution by absolute alcohol and ether. Saponin that now separates is detected by its sharp, harsh taste, foaming of the aqueous solution, behavior with concentrated sulphuric acid (githagin is soluble with yellow color gradually changing to purplish red), and haemolytic action. Further tests that may be employed for detection of githagin are the reducing action upon ammoniacal silver nitrate solution and Fehling's solution after previous boiling with hydrochloric acid.

Microscopic examination of the flour will also reveal presence of corn-cockle by the characteristic structure of the seed envelopes.

¹ K. Sagel. Detection of Saponin. Pharmazeutische Zentralhalle 55 (1914), 268.

Quantitative Estimation of Saponins in Plant Powders

(Korsakow¹)

This method is based upon the following observations 1 Saponins form unstable compounds with magnesium oxide 2 Saponins are soluble in boiling 80 per cent alcohol but insoluble in an ether-alcohol mixture 3 Saponin is not weighed as such but as sapogenin formed by hydrolysis of saponin

Procedure.—Make repeated extractions of the dry plant powder with boiling 60 per cent alcohol, filter the extract, distil off the alcohol, and evaporate the residue together with a little magnesium oxide to dryness upon the water-bath Powder the dry residue, exhaust it in a Soxhlet with boiling 80 per cent alcohol, filter, and precipitate saponin from the filtrate with ether Dissolve the precipitate in 3 per cent sulphuric acid, heat the solution for 1 hour in sealed tube to 105°, wash separated sapogenin upon the filter with water to neutral reaction, dissolve in absolute alcohol, expel alcohol, bring the precipitate to constant weight in vacuum desiccator, and weigh

SOLANINE AND SOLANIDINE

Solanine, $C_{28}H_{45}NO_{13}$, at the same time an alkaloid and glucoside (gluco-alkaloid) occurs in the potato plant (*Solanum tuberosum*) and in other Solanaceae such as *Solanum nigrum*, *Solanum dulcamara* and *Solanum lycopersicum* (tomato) It has been found also in Scopolaceae, such as *Scopolia orientalis* and *Scopolia atropoides* Solanine is not uniformly distributed in all parts of the potato plant but is most abundant in the berry-like fruit and in the chlorophyll-free sprouts appearing in the spring upon potatoes that lie in a cellar Schmiedeberg and Meyer found 0.024 gram of solanine per kilogram of peeled potatoes in January and February but 0.44 gram in unpeeled potatoes Potato peelings gave 0.71 gram of solanine per kilogram and potato sprouts 1 cm long even 5.0 grams New potatoes that have not wholly matured contain 5-6 times as much solanine as do those that have matured The same is also true of the black spots upon very old potatoes Wide-spread poisonings have repeatedly occurred as the result of eating such potatoes R Weil claims to have shown that two of the bacteria found in the black spots upon potatoes have the power of producing solanine Rather serious illness may appear in man from taking 0.3-0.4 gram of solanine with potatoes

Properties.—Solanine crystallizes in white needles having a bitter taste and melting at 244° Even boiling water dissolves only a little of this alkaloid (about 1:8000) It is soluble in 500 parts of cold and 125 parts of boiling alcohol, and in about 4000 parts of ether These solutions are faintly alkaline. Hot saturated solutions of solanine in alcohol and amyl alcohol gelatinize upon cooling Ether, chloroform and benzene do not extract solanine either from acid or alkaline solution But hot amyl alcohol extracts solanine from acid solution and from solutions alkaline with sodium peroxide or ammonia. Solanine is a weak base, readily dissolving in acids, such as acetic acid, forming crystalline salts Dilute hydrochloric or sulphuric acid hydrolyzes solanine to solanidine,

¹ M Korsakow Investigations of the Methods of Estimating Saponins. Comptes rend de l'Acad des Sciences 153 (1913), 844.